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Oxygen-Alkali Degradation of Loblolly Pine Dioxane Lignin:

Changes in Chemical Structure as a Function of Time of Oxidation

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OXYGEN-ALKALI DEGRADATION OF LOBLOLLY PINE DIOXANE LIGNIN: CHANGES IN CHEMICAL STRUCTURE AS A FUNCTION OF TIME OF OXIDATION

A thesis submitted by

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SUMMARY

The oxygen/alkali degradation of loblolly pine (Pinus taeda L.) dioxane lignin (DL) was investigated in terms of changes in functional group contents and molecular weight distributions with respect to time of oxidation. Oxidations were run in a Teflon-lined reactor using one gram samples of lignin in 50 ml of liquor. Reaction conditions were: temperature, 120°C; oxygen pressure, 0.618 MPa (89.7 psi, @ 120°C); alkali concentration, 0.13N or 0.50N Na_2CO_3.

Two oxidation series were run at 0.13N and 0.50N Na_2CO_3 to examine the role of alkali charge on structural changes. Model compound studies have indicated that free phenolic hydroxyl and α-carbonyl groups are involved in lignin degradation with oxygen/alkali. A sample of the DL was pretreated with peroxyacetic acid (PAA) to attempt to increase the content of free phenolic hydroxyl and α-carbonyl groups. The PAA-modified lignin was then oxidized with oxygen in 0.13N Na_2CO_3 as above and characterized. The chemical properties of the modified lignin were compared with those of the unmodified lignin oxidized with oxygen under similar conditions.

For all three oxidation series methoxyl contents of the oxidized lignins decreased with increasing time of oxidation. Phenolic hydroxyl and carbonyl contents decreased rapidly in the first hour of reaction, then remained essentially constant at longer oxidation times. Carboxylic acid contents increased rapidly during the first two hours of reaction, then leveled off at longer oxidation times. Gel permeation chromatography of the oxidized lignin indicated that, upon treatment of the lignin with oxygen/alkali, the lignin underwent extensive cross-linking in the initial stage of reaction to form a high molecular weight fraction which was subsequently degraded to low molecular weight products.

Pretreatment of the DL with PAA led to formation of carboxylic acids, loss of α-carbonyls, and formation of some low molecular weight material. Only a minor increase in phenolic hydroxyl (ca. 5%) was observed, suggesting that hydroxylated intermediates formed in the system were rapidly oxidized further to acidic products. Oxidation of the PAA-treated lignin with oxygen in 0.13N Na_2CO_3 and comparison of the analyses for the oxidized lignin with those for an unmodified lignin oxidized under similar conditions indicated no marked change in the reactivity of the modified lignin, with one exception, noted below.

The molecular weight distribution of a sample of the PAA-modified lignin oxidized for 8.0 hr with oxygen/alkali indicated that the high molecular weight fraction was much larger than the corresponding sample from the oxidation of the unmodified lignin. This result suggested that the PAA-modified lignin underwent more extensive cross-linking on charging the reactor with oxygen/alkali than the unmodified lignin. It was proposed that oxidation of the lignin with PAA led to greater mobility of the lignin fragments and, thus, increased access of resonance-stabilized radicals to coupling reactions.

Comparison of results for the lignin oxidized at two alkali concentrations indicated that lignin oxidized at the higher alkali concentration was more extensively degraded. Results suggested that the rate of lignin degradation was greatly reduced when the pH of the liquor declined to 9.4.

Based on the results of this investigation, it is postulated that oxidation of an isolated softwood lignin with oxygen in sodium carbonate leads initially to rapid cross-linking of the lignin to a high molecular weight fraction which subsequently degrades with time to low molecular weight

products. In addition, functional groups identified from model compound studies to be important in the initiation of lignin degradation, phenolic hydroxyl and α-carbonyl, were consumed during the first hour of reaction. These results suggest that degradation of the lignin after the first hour of reaction proceeded by secondary mechanisms.

INTRODUCTION

SIGNIFICANCE OF THE PROBLEM

The major process today for production of pulp is the kraft process, which accounts for approximately 70% (1) of wood pulp production in the United States. However, as a consequence of increased public and governmental concern over the quality of the environment, the chemical pulp industry has sought in recent years to develop a pulping process which eliminates, or at least reduces, the use of sulfur-containing pulping chemicals.

It has been known for more than half a century that molecular oxygen will degrade lignin (2). Recent success in the bleaching of conventional pulps with oxygen in alkaline solutions has led to interest in the development of oxygen/alkali (O/A) pulping. Cox and Worster (3) reviewed recent technology and concluded that the O/A pulping method offers the greatest potential for production of a marketable pulp by a sulfur-free process.

In order to overcome the problem of nonuniformity of delignification caused by low oxygen solubility and poor oxygen penetration, most O/A pulping processes employ a two-stage pulping scheme. Typically, the first stage involves a soda cook to a high yield followed by disk refining. In the second stage the high yield pulp is further delignified with oxygen in an alkaline solution.

The major deficiency of the oxygen pulping system is the significant degradation of carbohydrates which occurs concurrently with delignification to yield pulps with poorer strength properties than those produced by the conventional kraft process. Carbohydrate degradation in O/A pulping apparently proceeds by a radical mechanism (4). The radical decomposition may be

minimized, either by use of inhibitors, or by careful control of pulping parameters so that peroxide decomposition proceeds by an ionic rather than by a radical mechanism (5,6).

A third approach to minimizing carbohydrate degradation is to increase the susceptibility of the lignin to O/A degradation and thus reduce the time of exposure of carbohydrate to O/A pulping conditions. Thus, Marton and Leopold (7) found that a first stage soda cook of several conifers in the presence of oxygen at 10 psi gave a modified pulp which was more readily delignified during the second stage O/A cook than was the unmodified pulp. The improved delignification was attributed to preoxidation of the lignin during the first stage cook. Likewise, Johnson, et al. (8) observed that treatment of fiberized red maple with peroxyacetic acid (3-8% PAA, pH $\leq$ 7, o.d. wood basis) prior to O/A pulping led to faster and more selective delignification. However, a similar peroxyacetic acid pretreatment of fiberized loblolly pine failed to improve delignification under O/A pulping conditions.

In order to be able to modify the lignin in the wood to give increased reactivity, it is necessary to have a good understanding of the role of critical lignin functional groups toward reaction with O/A. This investigation is concerned with changes in functional group contents and molecular weight of an isolated lignin under O/A pulping conditions, as a function of time of reaction. A peroxyacetic acid-modified lignin was also examined in order to characterize the nature of structural changes in the lignin resulting from the pretreatment and the effect of these structural changes on the reactivity of the lignin toward degradation with O/A.

LIGNIN DEGRADATION WITH OXYGEN IN ALKALINE MEDIA[1]

In spite of wide interest in oxygen pulping and bleaching, there is still a good deal of uncertainty concerning the fundamental mechanisms of lignin degradation due to the complex nature of the oxidizing medium. Reaction patterns are obscured by the potential for alkaline hydrolysis, as well as the possible participation of strong oxidants such as hydrogen peroxide, hydroperoxy anions, and hydroperoxy, superoxide, and hydroxyl radicals formed in the stepwise reduction of oxygen. Although there is some evidence to suggest that lignin degradation by oxygen in alkali proceeds through an ionic mechanism (9,10), the majority of the evidence favors a radical mechanism (11-16). Both types of mechanisms may well play a role.

Probably the most significant work in the area of radical-initiated lignin degradation is that of Kratzl, et al. (11-13). The initial stage of reaction leads to formation of resonance-stabilized phenoxy and hydroperoxy radicals (Fig. 1). It is hypothesized that oxygen abstracts a single electron from a phenolate anion to yield a resonance-stabilized phenoxy radical and a hydroperoxy radical. Ionization of the phenol enhances the electron

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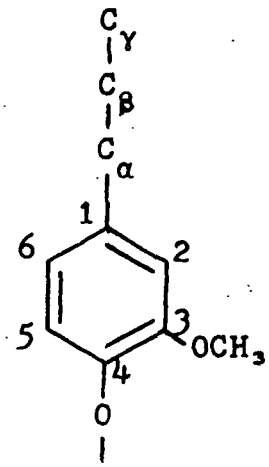
[1] Throughout this thesis, the following conventional notation for the carbon atoms of the guaiacylpropane structure of softwood lignin will be used.



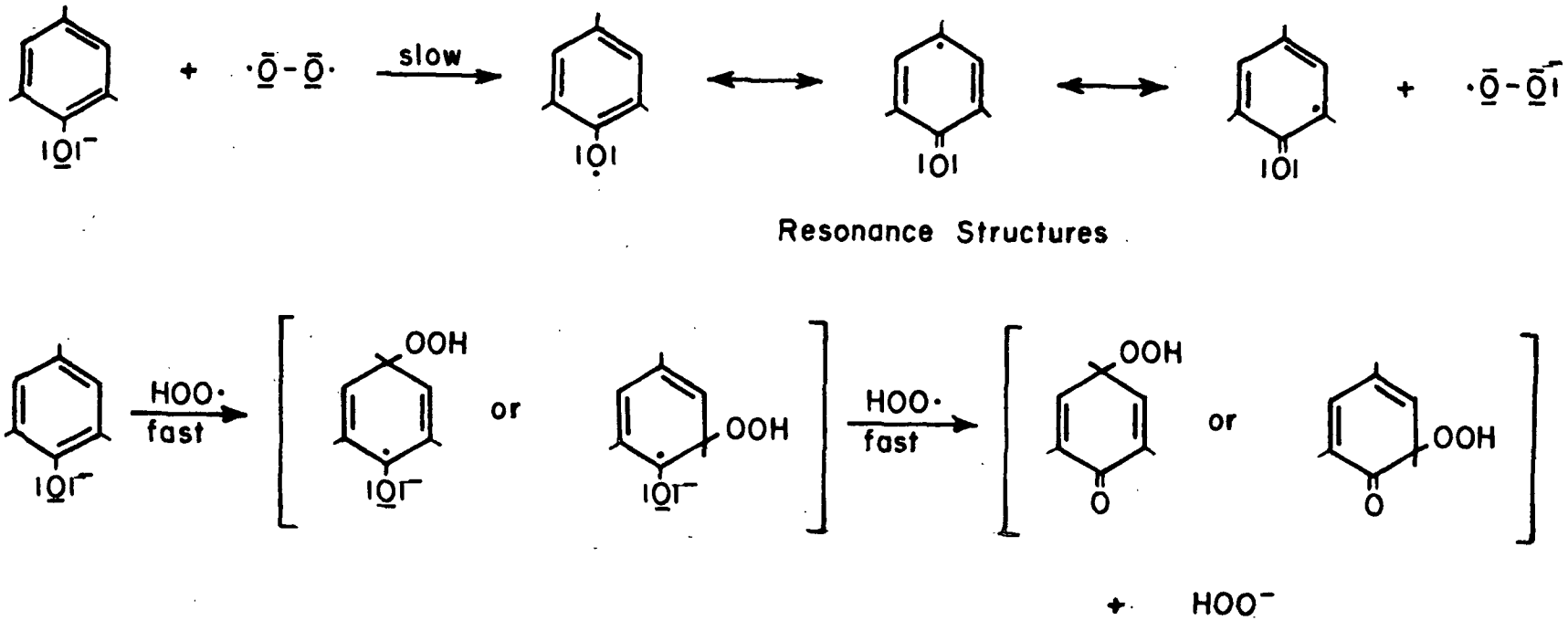


Figure 1. Primary Radical Reactions in the Oxygenation of Phenolic Structures (13)

abstraction. The hydroperoxy radical is a stronger oxidant than molecular oxygen and will react rapidly with additional phenolic structures to give either phenoxy radicals or, in a two-step reaction, cyclohexadienone hydroperoxides. The reaction between molecular oxygen and phenolate anion is thought to be the rate-controlling step in this mechanism.

Analysis of reaction products from degradation of lignin model compounds with oxygen in alkali has indicated a number of potential reaction pathways for subsequent degradation of phenolic moieties, summarized in Fig. 2 and 3.

1. Oxidative coupling of phenolic nuclei to form ortho-ortho-linked diphenols when the phenols are alkyl-substituted para to the phenolic hydroxyl group (11).
2. Side-chain elimination in model compounds having α-carbonyls and α-carbinols (12).
3. Formation of epoxidated quinol structures (14).

4,5. Ring cleavage between the methoxyl-bearing ring carbon and either of the two adjacent ring carbons (14).

Based on model compound studies it may be concluded that radical-initiated lignin degradation represents a competition between coupling of phenoxy radicals and formation of intermediate cyclohexadienone hydroperoxides. Kratzl, et al. (11) observed, for example, that oxidation (0.2N NaOH, 70°C) of creosol (2-methoxy-4-methylphenol) resulted in 60% of the creosol forming the o,o'-dimer. Dimerization was then followed by ring fission reactions to carbon dioxide, methanol, acetone, and acidic products. The intermediate cyclohexadienone hydroperoxides degraded further through side-chain elimination, demethoxylation, or ring-opening reactions.

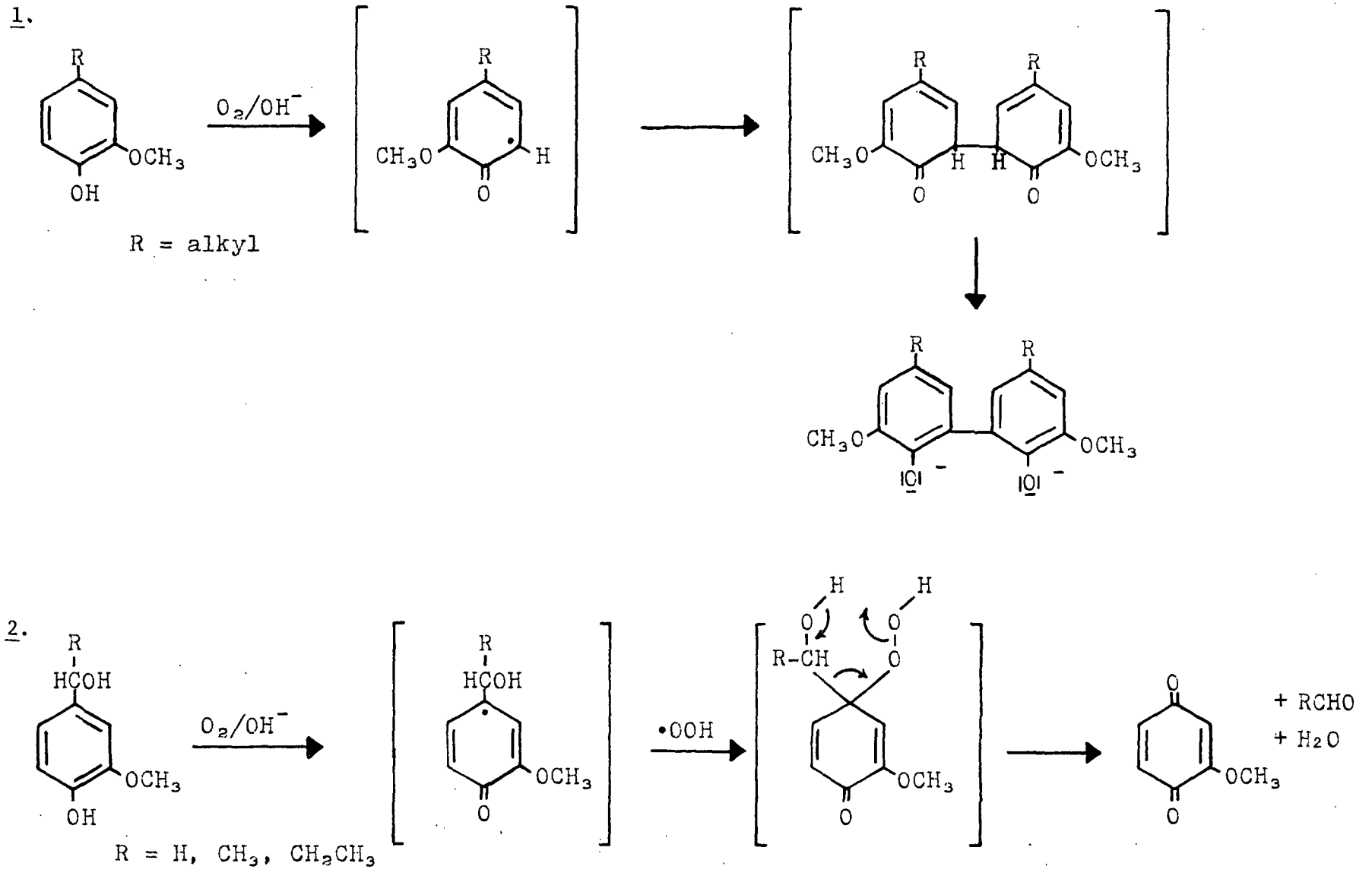


Figure 2. Proposed Mechanisms for the Radical-Initiated Degradation of Lignin Model Compounds with Oxygen in Aqueous Alkali (11,12)

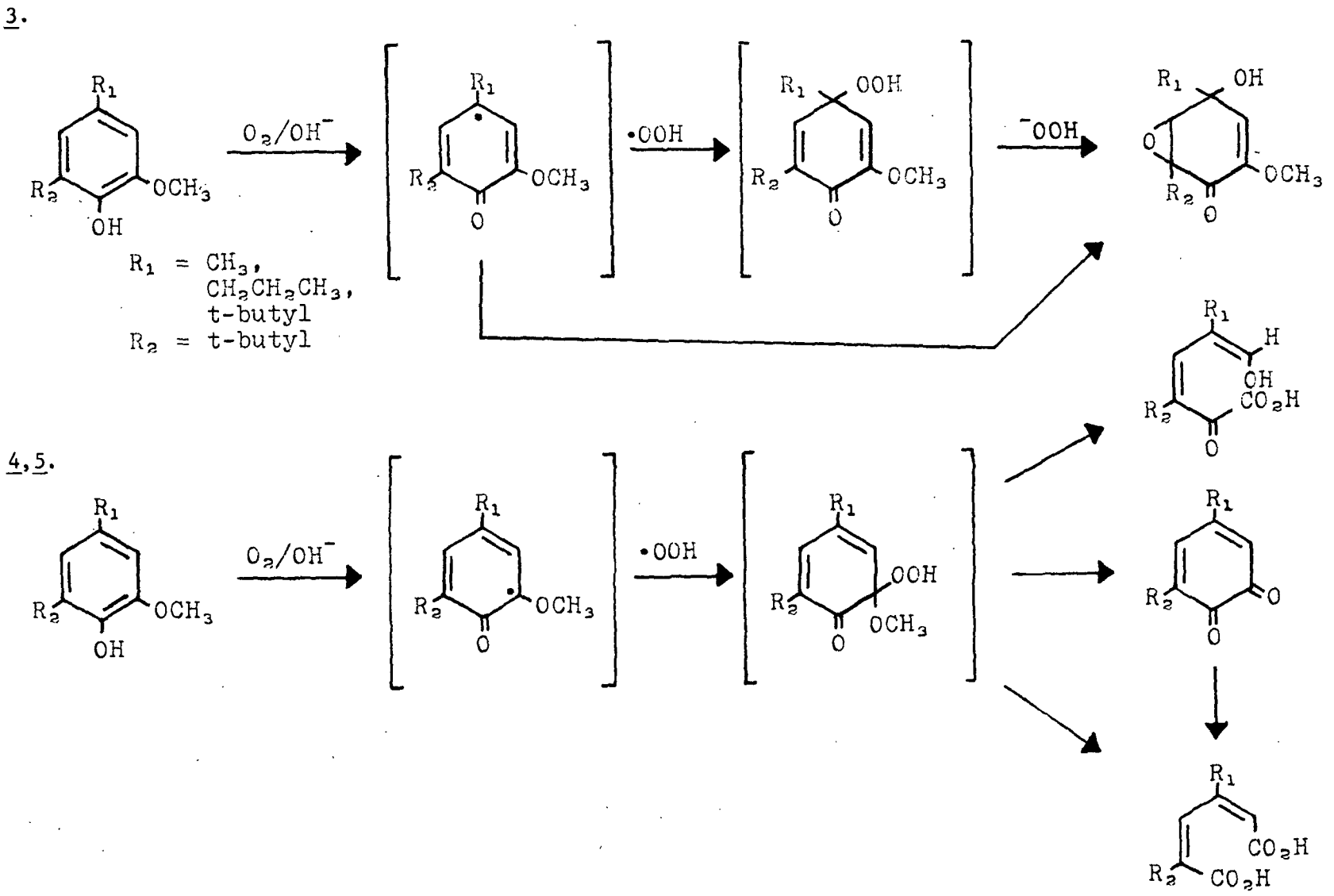
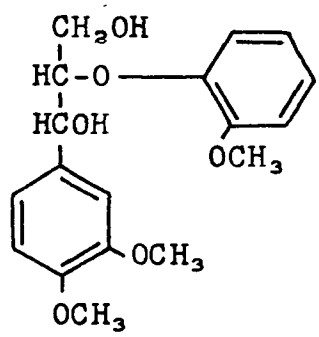


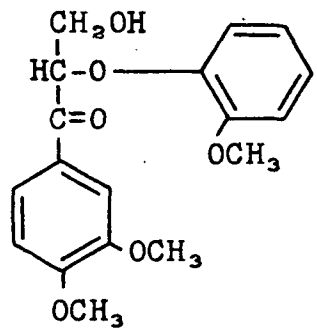
Figure 3. Proposed Mechanisms for the Radical-Initiated Degradation of Lignin Model Compounds with Oxygen in Aqueous Alkali (14)

Of the five mechanisms outlined in Fig. 2 and 3, only Mechanism 2 (Fig. 2) involves side-chain elimination which would lead to a decrease in molecular weight. Oxygenation of model compounds in which the phenolic hydroxyl is blocked with a methoxyl, but which possess an α-carbonyl group, have suggested the possibility of side-chain cleavage by an ionic mechanism (9,10).

Aoyagi, et al. (9) oxidized a number of lignin model compounds with oxygen in alkali. Whereas 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (I) was recovered essentially unreacted after 3 hours, 1-(3,4-dimethoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)-1-propanone (II) was reacted completely after 1 hour. Based on the results of the model compound studies,



I



II

it was proposed that the initiation step involved a base-catalyzed ionization of the β-carbon. The α-carbonyl increased the acidity of the β-methine proton and facilitated ionization. The general mechanism for degradation of lignin model compounds containing an α-carbonyl and no free phenolic hydroxyl is presented in Fig. 4. No specific mechanism for degradation of the hypothesized hydroperoxide intermediate was discussed.

A related study by Gierer, et al. (10) and Gierer and Imsgard (17) of lignin model compounds containing an α-carbonyl or a ring-conjugated olefin lends support to an ionic degradation mechanism. They likewise hypothesized

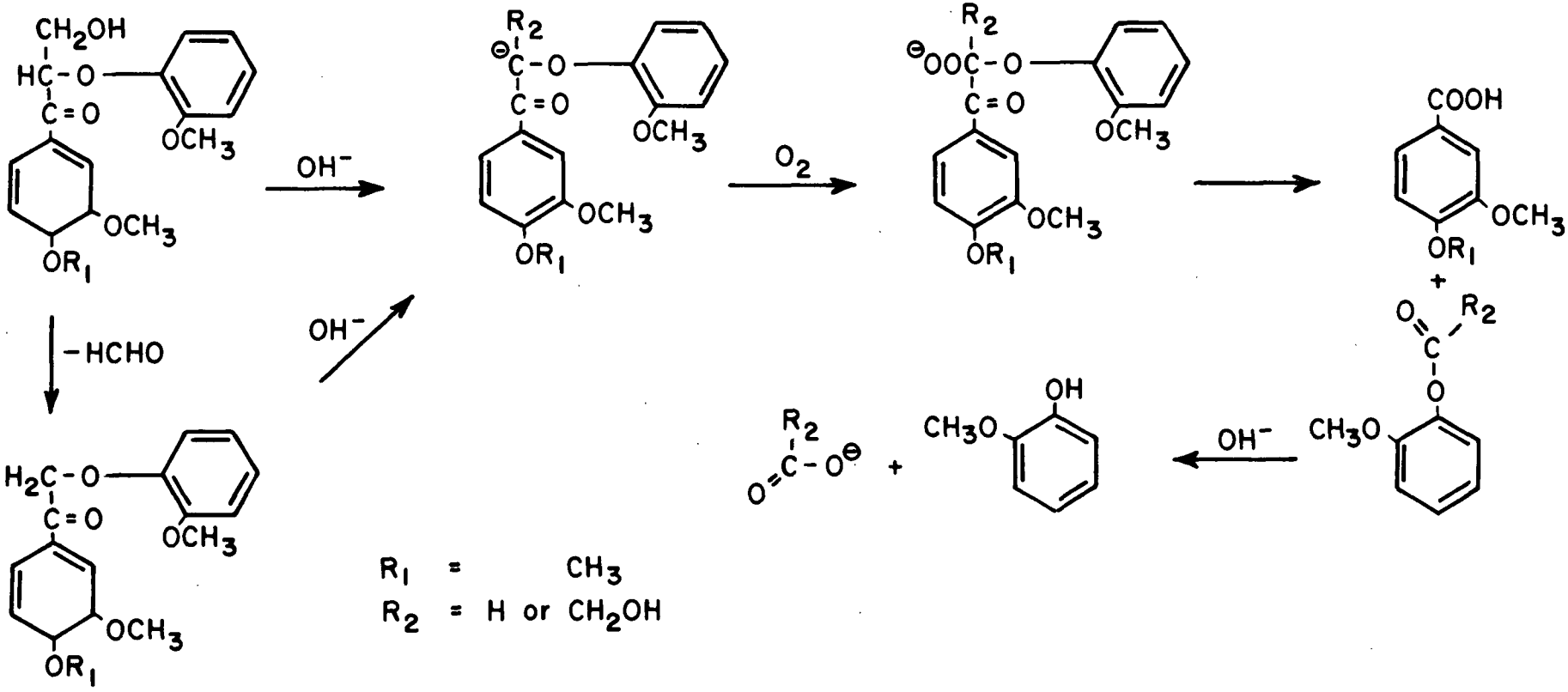


Figure 4. Proposed Mechanism for the Base Catalyzed, Ionic Degradation of Lignin Model Compounds with Oxygen in Aqueous Alkali (9)

a hydroperoxide intermediate. It was proposed that the hydroperoxide intermediates in both the carbonyl and ring-conjugated olefinic systems degraded ionically through a dioxetane intermediate (Fig. 5).

PEROXYACETIC ACID REACTIONS WITH LIGNIN

Based on proposed mechanisms for degradation of lignin model compounds with O/A, one would expect that an increase in phenolic hydroxyl and/or α-carbonyl content in lignin would result in an increase in the rate of lignin fragmentation. A reagent which has been reported to introduce these types of functional groups to lignin is peroxyacetic acid (PAA). PAA has also been found to be a highly selective oxidant for lignin in pulping and bleaching studies.

The reactions and chemistry of PAA have been reviewed in detail elsewhere (18). Results relevant to the present investigation will be discussed here.

Oxidation of guaiacyl systems with PAA indicates that reaction proceeds by electrophilic hydroxylation at activated positions of the phenolic ring system. Farrand and Johnson (19) studied the PAA oxidation of four guaiacyl-related model compounds. Major reaction pathways led to quinone formation and ring opening to give muconic acids and related lactones (Fig. 6).

In addition to attack of the aromatic ring, side-chain reactions may also occur, leading to formation of lower molecular weight products through cleavage of the β-aryl ether linkage. The β-aryl ether linkage is the most important link between C_9 units in the lignin macromolecular structure. Formation of an α-carbonyl precursor was proposed by Ishikawa, et al. (20) in the cleavage of β-aryl ethers in model compound studies (Fig. 7).

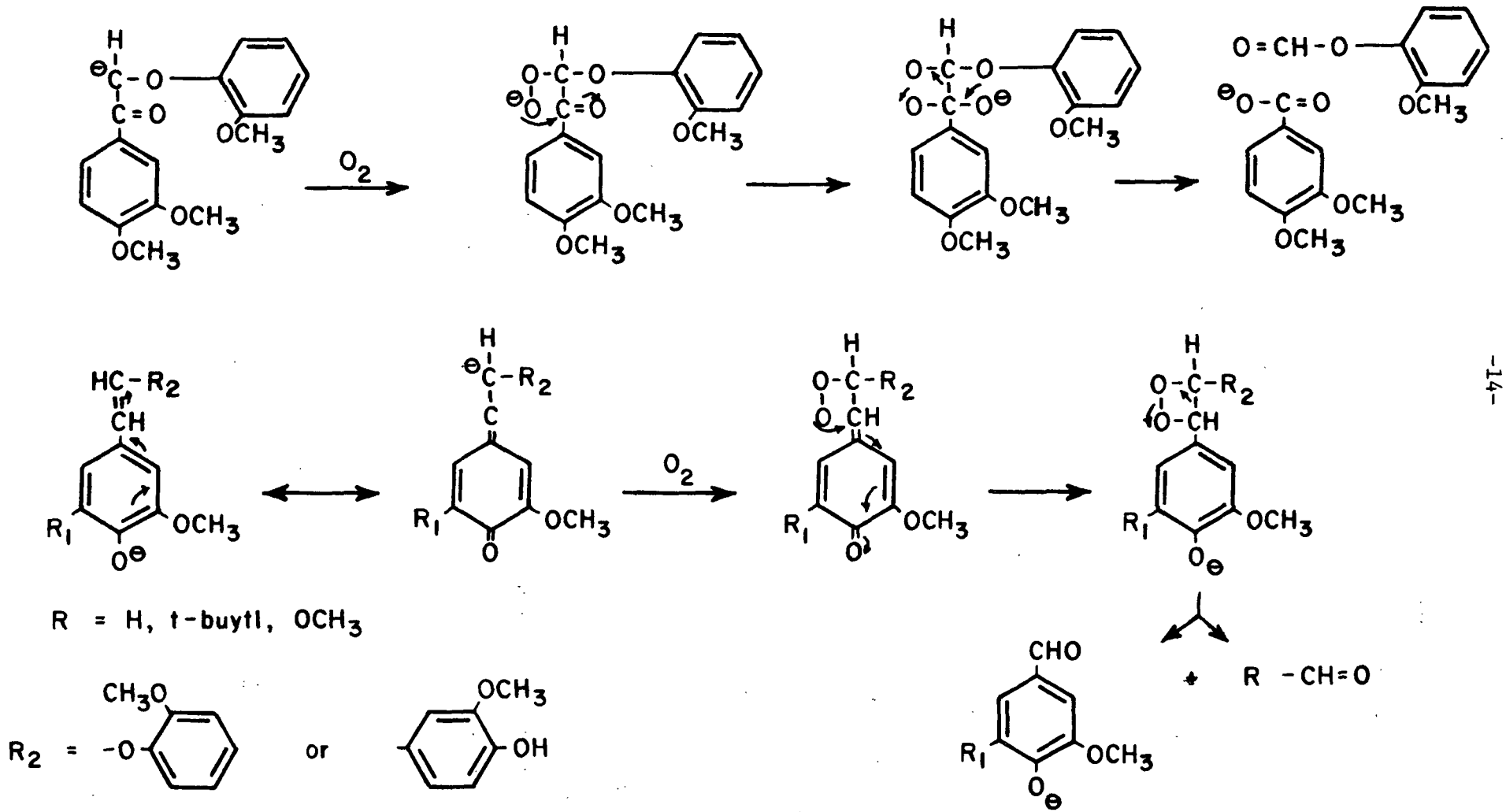


Figure 5. Proposed Mechanism for the Base Catalyzed, Ionic Degradation of Lignin Model Compounds with Oxygen in Aqueous Alkali via Dioxetanes (10)

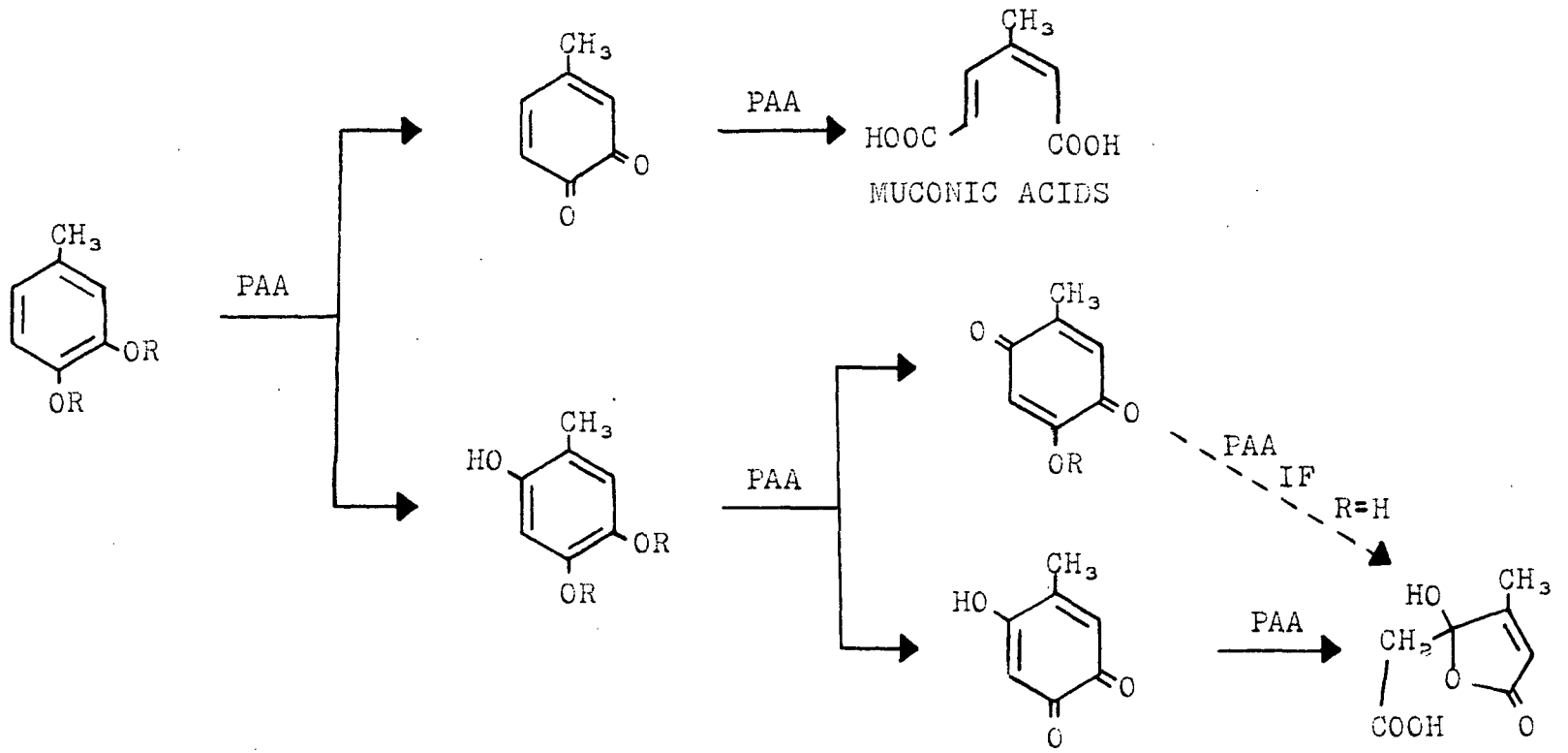


Figure 6. Oxidation Pathways for Peroxyacetic Acid Reaction with 4-Methylpyrocatechol Derivatives (19)

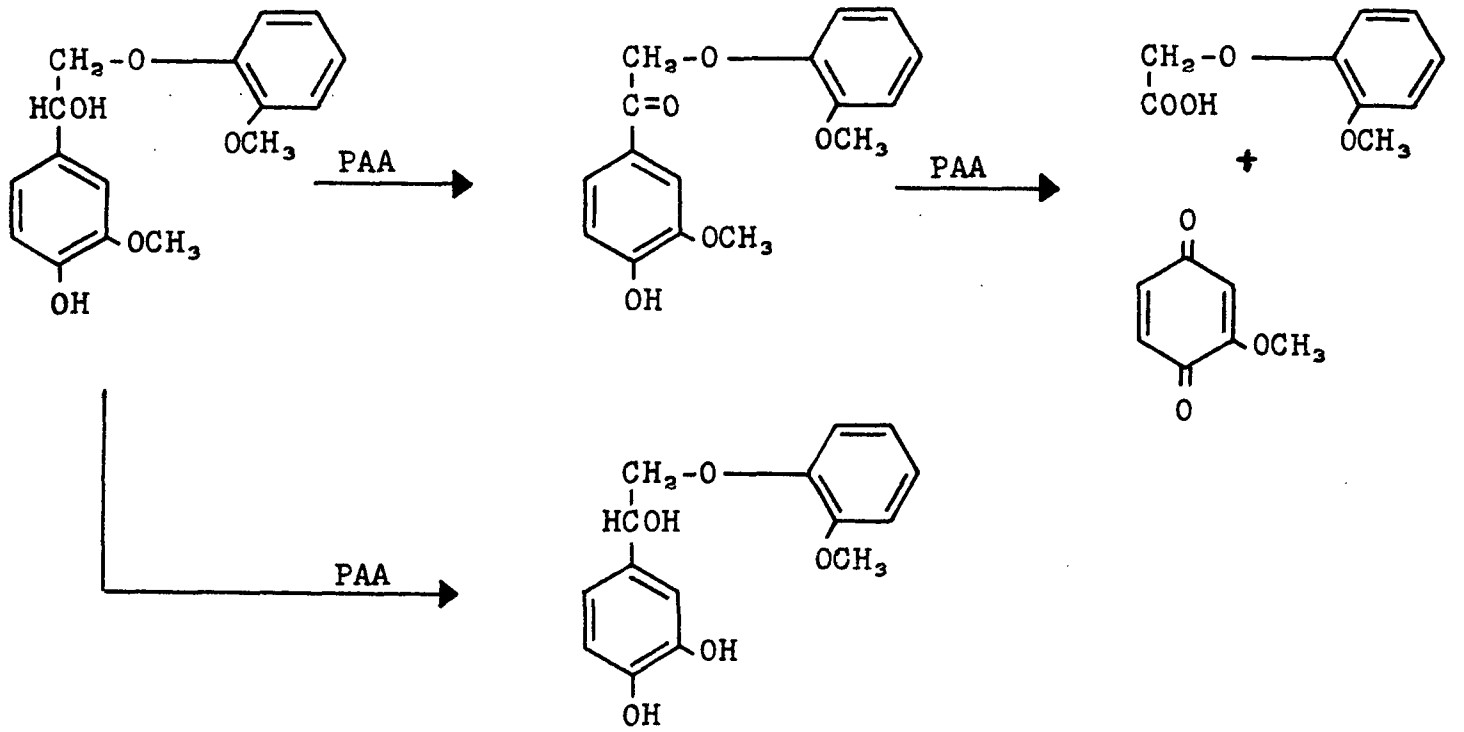


Figure 7. Reaction of Peroxyacetic Acid with a β-Aryl Ether (20)

The importance of the α-carbonyl group was confirmed in another study by Sakai, et al. (21) in which they investigated the behavior of β-aryl ether linkages in lignin model compounds under mild PAA oxidation conditions. It was observed that compounds with an α-hydroxyl group in the side chain were less reactive than those containing an α-carbonyl group. Oxidation of the β-aryl ether bond of softwood model compounds proceeded readily and gave rise to guaiacol or its acetate. In a subsequent study (22) splitting of the β-aryl ether linkage in pine and birch milled wood lignins (MWL) was found to play an important role in lignin solubilization during PAA oxidation.

Sakai and Kondo (23) oxidized dioxane lignin (DL) from pine and birch with PAA and observed a correlation between rate of demethylation and solubilization of the lignin. They proposed that the rate determining step of the degradation involved loss of methoxyl.

Of particular interest to the present investigation was a study in which Ishikawa, et al. (24) oxidized a DL from a pine groundwood pulp and separated the degraded product into water-soluble and water-insoluble fractions. Based on this study, it was proposed that degradation led to formation of muconic acids, side-chain oxidation, demethoxylation, increased phenolic OH and COOH contents, and formation of catechol structures which were readily oxidized to quinones. An increase in phenolic OH and COOH contents of the soluble portion of pine lignin upon PAA treatment has likewise been reported by Albrecht (18,25).

PREPARATION OF ISOLATED LIGNINS UNDER MILD CONDITIONS

For the purposes of this investigation it was desired to isolate a lignin fraction from a wood sample which could be collected in sufficient

yield for extensive chemical study, but which might be considered representative of the lignin as it existed in the wood.

Milled wood lignins (MWL) and cellulolytic enzyme lignins (CEL) are considered to be the best lignin preparations now available (26). The most widely used method for preparation of a mildly isolated lignin has been that of Bjorkman (27-29) which employs extensive vibratory ball milling of the wood in a toluene suspension. Milling times are typically several weeks. Extraction of the wood meal with a 90% (v/v) solution of aqueous dioxane gives a lignin at a yield of approximately 30% of the Klason lignin content. The "Bjorkman lignin" or MWL typically contains a few percent carbohydrate, but modifications of the procedure by Lundquist and Simonson (30) and Lundquist, et al. (31) have lowered the carbohydrate level to as little as 0.05%.

Pew (32) and Pew and Weyna (33) reported that wood which had been subjected to 5-8 hours of milling in a vibratory ball mill could be freed of most of its saccharides by treatment with a glycosidase. However, the residual lignin was found to contain 12-14% carbohydrate. Chang, et al. (34) milled wood for 48 hours and treated the meal with an enzyme preparation containing high cellulolytic and hemicellulolytic enzyme activities. Two fractions of CEL were isolated by successive extraction with 96 and 50% (v/v) aqueous dioxane. Lignin yields from spruce were 27.8 and 29.2% of the Klason lignin, with carbohydrate contents of 4.3 and 8.9% for the respective samples.

For the present investigation neither the MWL nor the CEL appeared to be particularly well suited. Because of the length of time required for ball milling, the MWL procedure would have been prohibitively long to obtain enough lignin for the study. Although the milling time was significantly

reduced in CEL methods, the procedure was still lengthy and gave a lignin with relatively high carbohydrate contents which would interfere with lignin functional group analyses.

Therefore, a third type of lignin was prepared according to the procedure of Pepper, et al. (35) and Pepper and Siddiqueullah (36), known as dioxane lignin (DL), which has been shown to possess functional group characteristics similar to MWL (37). Yields of recovered lignin have been reported to be comparable to MWL preparations with somewhat lower carbohydrate contents. The preferred terminology, according to Lai and Sarkanen (38), would be dioxane acidolysis lignin to emphasize potential hydrolysis reactions which may be associated with the isolation. The DL is prepared by refluxing Wiley-milled wood meal for 1 hour with a 90% (v/v) aqueous dioxane solution containing 0.2N HCl.

It must be recognized that due to degradation of the lignin in the isolation procedure, the isolated lignin is not the same as the in situ lignin. As a result of ball milling of the wood and fragmentation of the lignin, the molecular weight of 15,000 for a spruce MWL (34) is certainly much lower than that of the original lignin. Additionally, increased free phenolic hydroxyl and α-carbonyl contents have also been found to be associated with the ball milling process (32,34), the magnitude of the increase being directly related to the length of time of milling.

In addition to the structural changes noted above, preparation of DL according to the procedure of Pepper, et al. (35) and Pepper and Siddiqueullah (36) will be expected to lead to hydrolysis of acid labile bonds in the lignin. The major hydrolysis reaction appears to be cleavage of the benzyl aryl ether linkage, resulting in lower molecular weight and increased phenolic hydroxyl content (39).

THESIS OBJECTIVE AND EXPERIMENTAL APPROACH

The objective of this thesis was to gain a better understanding of the role of various important functional groups in the degradation of lignin with O/A. The investigation sought to bridge a gap in the literature between model compound studies which have been concerned with mechanisms of lignin degradation in O/A and pulping and bleaching studies which have been primarily concerned with process optimization.

The experimental approach involved isolation and characterization of dioxane lignin (DL) prepared from loblolly pine. The isolated lignin was next oxidized under O/A pulping conditions and the changes in functional groups characterized as a function of time of reaction.

Based on a review of proposed mechanisms for degradation with O/A, phenolic hydroxyl and α-carbonyl groups appeared to be important in initiation of lignin degradation. Peroxyacetic acid (PAA) has been shown in model compound studies to degrade lignin through formation of intermediate phenolic hydroxyls by hydroxylation of the aromatic ring and through intermediate formation of α-carbonyls by oxidation of α-carbinols. Therefore, a sample of DL was chemically modified by treatment with a limited amount of PAA. The PAA-modified lignin was characterized and then oxidized with O/A. The degraded PAA lignin was characterized for changes in functional groups as a function of time of reaction. Results were compared with the O/A oxidation of an unmodified lignin oxidized under similar reaction conditions.

RESULTS AND DISCUSSION

ISOLATION AND CHARACTERIZATION OF DIOXANE LIGNIN STARTING MATERIAL

Approximately 140 g of DL was isolated from loblolly pine (Pinus taeda L.) by repeated extraction of 100 g samples of preextracted (acetone) wood meal with 90% (v/v) aqueous dioxane containing 0.2N HCl according to a procedure modified from Pepper, et al. (35,36). The precipitated DL was freeze dried and then homogenized by combining all of the DL in a mortar and grinding together with a pestle to a fine powder. Klason lignin content of the extracted wood meal was 30.63%. Yield of DL ranged from 11.5 to 32.8% with an average yield of 20.6% of the Klason lignin. Arseneau and Pepper (37) isolated 11.8% of the Klason lignin from spruce as DL by a similar procedure. Conditions for isolation of the DL in the present investigation were the same for each 100 g sample of wood meal and no explanation for the wide variation in yields could be found. Following homogenization, the DL was partitioned into polyethylene bottles and stored under vacuum over P_2O_5 at 40°F until needed for further analysis.

There is little information in the literature on the chemical composition of loblolly pine DL. In general, however, the chemical composition of mildly isolated softwood lignins has been found to be fairly constant, regardless of species. As has been mentioned in the Introduction, the best lignin preparations available are MWL and CEL. Therefore, literature values for the chemical composition of these mildly isolated softwood lignins will be used as a reference for comparison with values for the DL used in this investigation.

Extensive chemical characterization of the homogenized DL was carried out for three reasons: first, to demonstrate that the lignin was in fact homogeneous with respect to all properties of interest; second, to demonstrate

that the DL was representative of isolated softwood lignins by comparison of values for chemical properties with literature values for mildly isolated softwood lignins; and third, to provide a well characterized starting material which would be used for comparison in later work with lignins isolated following oxidative treatments.

ASH AND MOISTURE CONTENTS

An average ash content of 3.1% (determined as the oxide ash) was found for the DL. Arseneau and Pepper (37) reported an ash content of 0.09% for spruce DL. Fleck (40) reported an ash content of 0.26% for loblolly pine DL. Yield of DL isolated for this investigation was significantly higher than yields reported by Arseneau and Pepper (37) and the higher ash content may have been associated with the higher yield through some type of coprecipitation of the inorganic material upon isolation of the DL. The sodium content of the lignin was 1.0%, determined as the sulfated ash. It seems likely that the ash was principally sodium sulfate, which was used in the precipitation step of the isolation procedure. No attempt was made to reduce the ash by dialysis as it was felt this procedure would lead to loss of lower molecular weight lignin. Chemical analyses of the DL were corrected for an ash content of 3.1%.

Following homogenization, the DL was stored in a desiccator under vacuum over P_2O_5. Determination of oven-dry (o.d.) weight (110°C, 24 hr) indicated an average moisture content of 5.64% in the DL, even after storage for several months. Therefore, chemical analyses of the DL were corrected for moisture content.

METAL CONTENT

A number of authors (5,6,41-47) have reported that trace levels of certain metal ions will have pronounced effects on delignification and/or carbohydrate degradation of oxygen pulps. The stabilization of carbohydrates in oxygen pulping by addition of magnesium salts has already been mentioned (5,6). Several studies have appeared on the detrimental effects of certain transition metal ions in oxygen bleaching (41-44). Landucci and Sanyer (45,46) studied the effect of a number of transition metal ions in oxygen pulping and found only manganese to be beneficial in that it increased pulp yield, pulp viscosity, and delignification rate. Other transition metals were found to either decrease the rate of delignification (Fe, Co, and Ni) or to increase the rate of carbohydrate degradation (Cu, Fe, and Co). Karna (47) reported that molybdate accelerated the O/A degradation of lignin in Douglas-fir wood meal.

The effect of transition metal ions on delignification rates is extremely complex and was not dealt with in this investigation. However, every effort was made to minimize metal contamination of the DL and oxidation liquors. In addition, metal contents of the DL starting material and selected oxidized lignin samples were measured in order to have some idea of the level of various critical metals in the reaction systems. Results of the metal analyses are summarized in Appendix II.

In the oxidation of the DL with O/A, a gram of DL was slurried in 50 ml of triply distilled water. Thus, as an example, the iron concentration of 0.015% (Table XXXI, Appendix II) was equivalent to a concentration in solution of 0.003 g/liter or 3 ppm. While it is difficult to relate the present investigation to the pulping studies of Landucci and Sanyer (45,46), a value

of 3 ppm was in the same concentration range used by these workers in which they did observe catalytic effects for iron in the oxygen pulping of softwoods. The present investigation was concerned with relative changes in functional groups as a function of time of reaction. Catalytic levels of metal ions should have been comparable for all samples in a given oxidation series since equivalent amounts of lignin, water, and alkali were used. Therefore, comparison of changes in functional groups within a given series should have been valid.

CARBOHYDRATE CONTENT

An average carbohydrate content of 1.4% was found for the DL, determined by gas chromatographic analysis of the alditol acetates prepared from the hydrolyzed lignin (48) (Table I). Chang, et al. (34) reported a carbohydrate content of 4.1% for spruce MWL and 4.3 and 8.9% for enzymatically liberated spruce lignin. Rezanowich, et al. (49) found carbohydrate contents of 0.05% for both MWL and DL isolated from spruce. Wide variation in carbohydrate content, even in lignins isolated from similar sources under similar procedures, may represent preparative differences.

For this investigation, chemical analyses of the DL starting material were corrected for a 1.4% carbohydrate content. It was felt the correction would be small enough to ignore in the samples from the oxidation studies discussed later.

ELEMENTAL ANALYSIS AND METHOXYL CONTENT

Fundamental analytical data for the DL are presented in Table II. Using the data from Table II, an empirical formula was calculated, based on the widely accepted C_9 unit structure for lignin. The result is tabulated in Table III and compared with structural formulas computed for other mildly

TABLE I

CARBOHYDRATE CONTENT OF DIOXANE LIGNIN

Monosaccharide	Analysis[a,b,c], % Sample 1	Sample 2
Araban	0.12	0.13
Xylan	0.36	0.35
Mannan	0.45	0.42
Galactan	0.39	0.36
Glucan	0.15	0.13
Total	1.47	1.39

[a] Based on o.d. weight.

[b] Corrected for an ash content of 3.1%.

[c] Average of duplicate determinations, sample numbers refer to two separate aliquots of DL.

TABLE II

METHOXYL AND ELEMENTAL ANALYSIS OF DIOXANE LIGNIN

	Analysis[a,b,c,d], %
Methoxyl	15.99
Carbon	63.43
Hydrogen	5.73
Oxygen	30.84

[a] Based on moisture-free (dried over P_2O_5/vacuum) weight.

[b] Corrected for an ash content of 3.1%.

[c] Corrected for a carbohydrate content of 1.4%, assuming a structure of $C_6H_{12}O_6$.

[d] Average of triplicate determinations.

isolated softwood lignins. The empirical formula calculated for the DL used in this study was in good agreement with the other formulas for mildly isolated softwood lignins, lending support to the choice of DL for the investigation.

TABLE III

EMPIRICAL FORMULAS FOR MILDLY ISOLATED SOFTWOOD LIGNINS

Softwood Lignin	Structural Formula				Formula Weight	Reference
	C	H	O	(OCH_3)		
Loblolly pine DL	9	7.81	2.67	0.97	188.9	This work
White spruce DL	9	8.45	2.37	1.02	186.2	(37)
Scotch pine MWL	9	8.54	2.53	0.95	186.7	(28)
Norway spruce MWL	9	8.35	2.45	0.96	185.5	(28)
Norway spruce MWL	9	8.05	2.84	0.95	191.1	(34)
Norway spruce CEL	9	7.92	3.11	0.96	195.7	(34)

ULTRAVIOLET SPECTROSCOPY

Ultraviolet absorption of lignin systems is associated with transitions of nonbonded and π-bonded electrons. The location and intensity of these bands depend, however, on the nature of the substitution. Thus, a number of workers (50-54) have examined UV spectra of lignin model compounds in an attempt to relate the principal UV bands of lignin to specific structural features. Szabo-Lin and Teder (55) resolved the UV spectrum of spruce MWL into 14 bands. Spectra obtained in neutral or acidic solutions could be resolved by applying 10 overlapping bands, while spectra run at a pH of 13 required 14 overlapping bands. Although assignment of lignin structural features based on UV spectra alone is a questionable practice, when used in conjunction with other analyses, UV spectra can confirm or provide additional information concerning the nature of structural changes in lignin.

Two types of UV spectra can be defined: direct and difference. A direct spectrum is obtained by placing a solution of the sample in the sample beam of the spectrophotometer and a solvent blank in the reference beam. To obtain a difference spectrum an aliquot of the sample is made alkaline and placed in the sample beam of the instrument. The difference spectrum is then run by comparison with an acid or neutral solution of the sample placed in the reference beam.

Direct Ultraviolet Spectrum

Literature on the UV spectra of lignin has been reviewed by Goldschmid (56). The direct UV spectrum of the DL (Fig. 8) exhibits the typical features seen in UV spectra of lignin preparations. The spectrum decreases from a maximum at about 205 nm through a shoulder at 230 nm, to a characteristic lower maximum at 280 nm, followed by a gradual decrease toward the visible region of the spectrum.

Although the major absorption band near 205 nm is generally assigned to electronic transitions of substituted benzene rings, a number of acidic and ester structures may also absorb in this region. The shoulder at 220-230 nm is likewise generally thought to be related to electronic transitions of substituted benzenes.

Free and etherified phenolic hydroxyl groups are major contributors to the band at 280 nm. The intensity of the absorption in this region is related to the extent of oxygen substitution on the aromatic ring. Demethoxylation leads to a shift of this band to lower wavelength and a decrease in intensity of the band (16). The broad absorption in the region above 300 nm has been attributed to chromophoric groups conjugated to the aromatic ring, such as α-carbonyl and α-ethylenic structures.

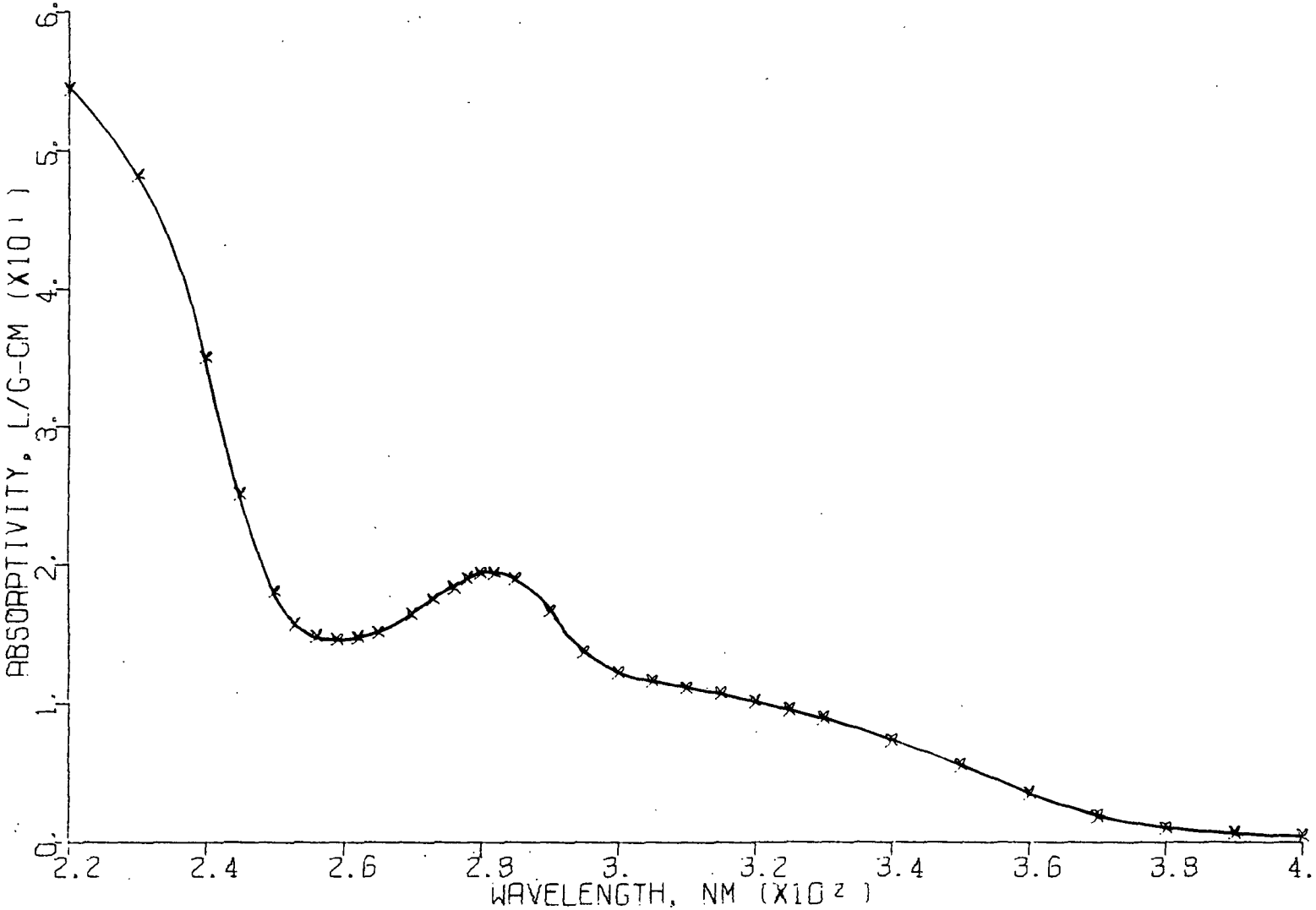


Figure 8. Direct Ultraviolet Spectrum of Dioxane Lignin

Two structural changes which may be anticipated to result from oxidation of the lignin and which may be expected to affect the UV spectrum are formation of muconic acids and biphenyl linkages. α-, β-Unsaturated carboxylic acids absorb very strongly in the 220-230 nm region (57) and thus, oxidation of lignin to muconic acids would be expected to introduce absorption bands in this region. Pew (58) reported that lignin-related, 5,5'-linked biphenyl structures had a major absorption band at 280 nm with high absorbance in the 250 and 300 nm regions, relative to the corresponding compounds containing a single aromatic ring. Therefore, formation of biphenyl linkages in the oxidation of the lignin would be expected to increase the absorbance of the oxidized lignin in the 250, 280, and 300 nm regions.

The absorption coefficient relating absorbance at 280 nm to concentration was 20.6 liters-g^{-1}-cm^{-1} for the DL. This value was identical to a value of 20.6 liters-g^{-1}-cm^{-1} reported for spruce MWL (28). Rezanowich, et al. (49) reported values of 19.5 liters-g^{-1}-cm^{-1} for spruce MWL and 19.8 liters-g^{-1}-cm^{-1} for spruce DL.

Ultraviolet Difference Spectrum

The UV difference spectrum for the DL (Fig. 9) exhibits the typical features seen in UV difference spectra of lignin preparations. Assignments of the absorption bands have been summarized by Goldschmid (56) (Table IV). The UV difference technique was originally developed to study the effects on the lignin UV spectrum of ionized phenolic hydroxyl groups (59,60).

Typical UV difference spectra of lignin materials pass through minima at about 230, 280, and 320 nm and through a major maximum at 250 nm, a lower maximum at about 300 nm and a broad maximum between 340 and 380 nm. The maxima at 250 and 300 nm are thought to correspond to electronic transitions

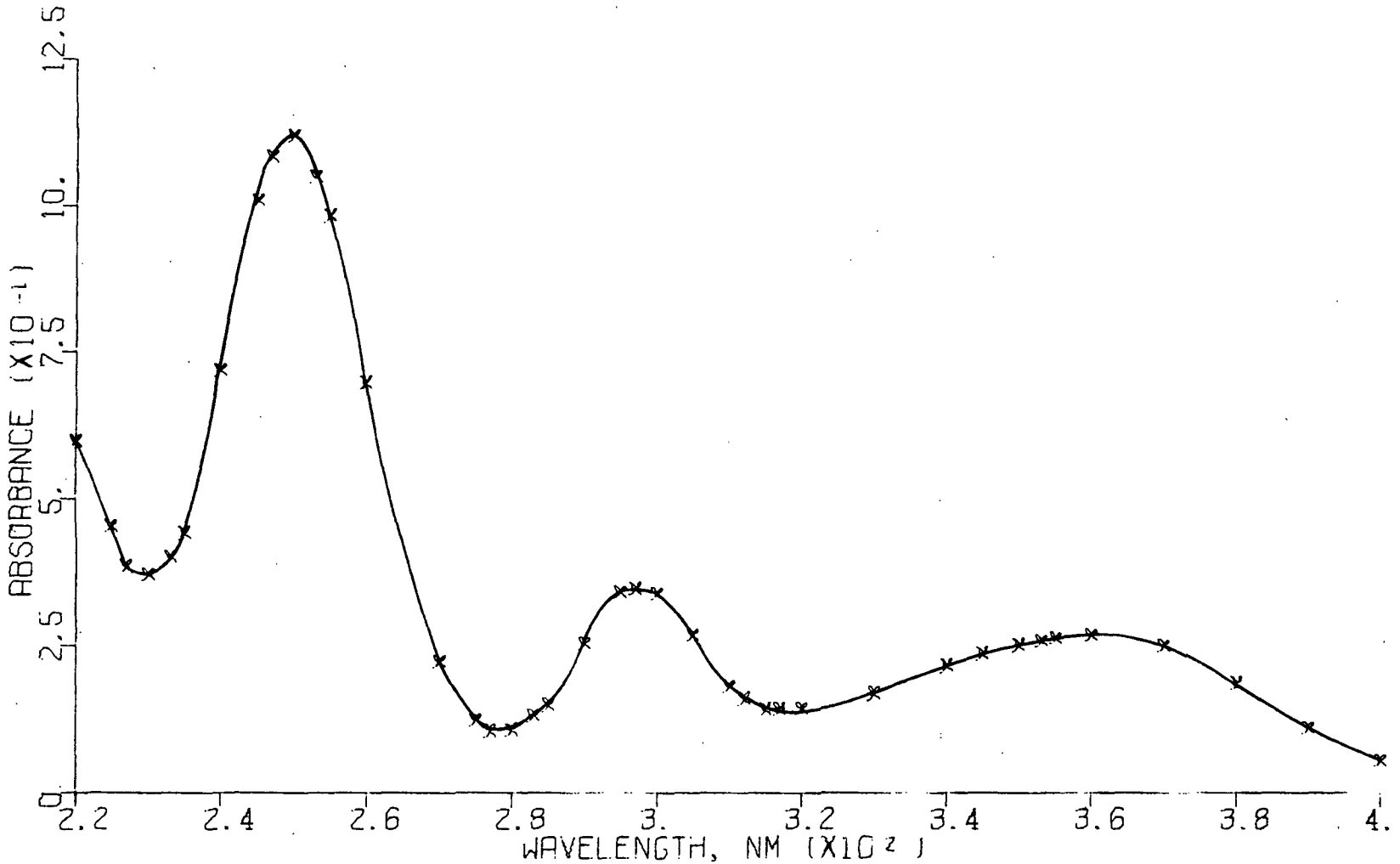
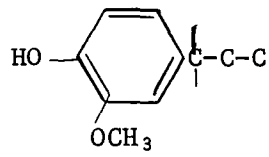
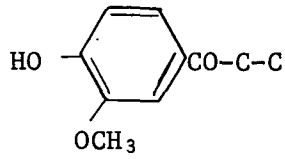
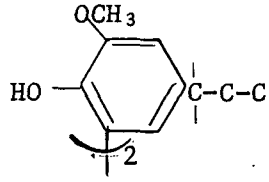
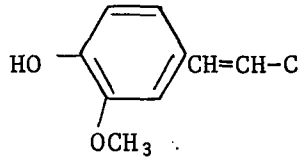
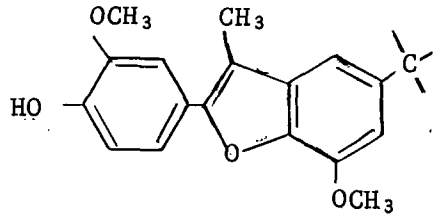
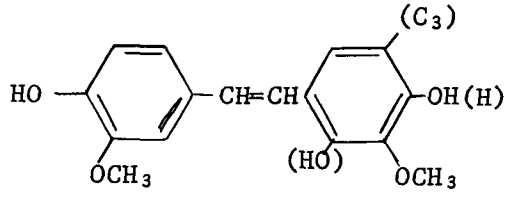
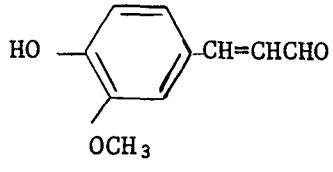


Figure 9. Ultraviolet Difference Spectrum of Dioxane Lignin

TABLE IV

ULTRAVIOLET BAND ASSIGNMENTS FOR SOFTWOOD LIGNIN STRUCTURES (56)

Structure	Approximate λ_{max}-values, nm	
	Nonionized or Etherified	Ionized
	230 280	250 300
	240 280–310	250 350
	220 250 290	230 265 310
	260 300	290 320
	310	330
	330	370–380
	250 350	260 400

of the benzene ring shifted toward longer wavelengths as a result of the ionization of phenolic hydroxyl groups in alkaline solution. The broad maximum between 340 and 380 nm is thought to be associated with α-carbonyls and α-ethylenic groups conjugated to an aromatic ring containing a free phenolic.

INFRARED SPECTROSCOPY

Infrared spectroscopy has been extensively applied to studies of functional group changes in chemically modified lignins (37,61-70). Band assignments for infrared spectra of isolated lignins have been reviewed by Hergert (71) (Table V).

The IR spectrum of the DL was obtained using KCl wafers (Fig. 10). Potassium chloride was used rather than potassium bromide to reduce absorption due to the presence of moisture. The major absorption bands reported by Hergert (71) (Table V) can all be identified in the DL. Comparison of the spectrum of DL with spectra of softwood MWL shows excellent agreement, with the exception of the band at 1715 cm^{-1}, which is more intense in the DL spectrum (64). A peak of similar intensity at 1715 cm^{-1} was likewise observed by Fleck (40) in the IR spectrum of loblolly pine DL.

Hergert (71) reported that along with an increase in the intensity of the 1715 cm^{-1} band, the band at 1660 cm^{-1} could be rationalized on the basis of acidic degradation of β-aryl ether linkages to β-ketones. However, based on model compound studies, the β-aryl ether linkage is stable toward hydrolysis under conditions used for isolation of the DL (39).

Reduction of the DL with sodium borohydride according to the method of Sarkanen, et al. (64) resulted in the disappearance of the 1660 cm^{-1} band,

TABLE V

ASSIGNMENTS OF INFRARED ABSORPTION BANDS
IN MILDLY PREPARED SOFTWOOD LIGNINS (71)

Position, cm^{-1}	Band Origin
3425-3400	OH stretching (H-bonded)
2920	CH-stretch in methyl and methylene groups
2875-2850	CH-stretch in methyl and methylene groups
2820	CH-stretch in methyl and methylene groups
1715	Carbonyl stretching-unconjugated ketone and carboxyl groups
1675-1660	Carbonyl stretching-para-substituted aryl ketone
1605	Aromatic skeletal vibrations
1515-1510	Aromatic skeletal vibrations
1470	C-H deformations (asymmetric)
1460	C-H deformations (asymmetric)
1430	Aromatic skeletal vibrations
1370	C-H deformation (symmetric)
1270	Guaiacyl ring breathing with CO-stretching
1140	Aromatic C-H in-plane deformation
1085	C-O deformation, secondary alcohol and aliphatic ether
1035	Aromatic C-H in-plane deformation and C-O deformation, primary alcohol
970	=CH out-of-plane deformation (trans)
855	Aromatic C-H out-of-plane deformations
815	Aromatic C-H out-of-plane deformations
750-770 shoulder	Aromatic C-H out-of-plane deformations

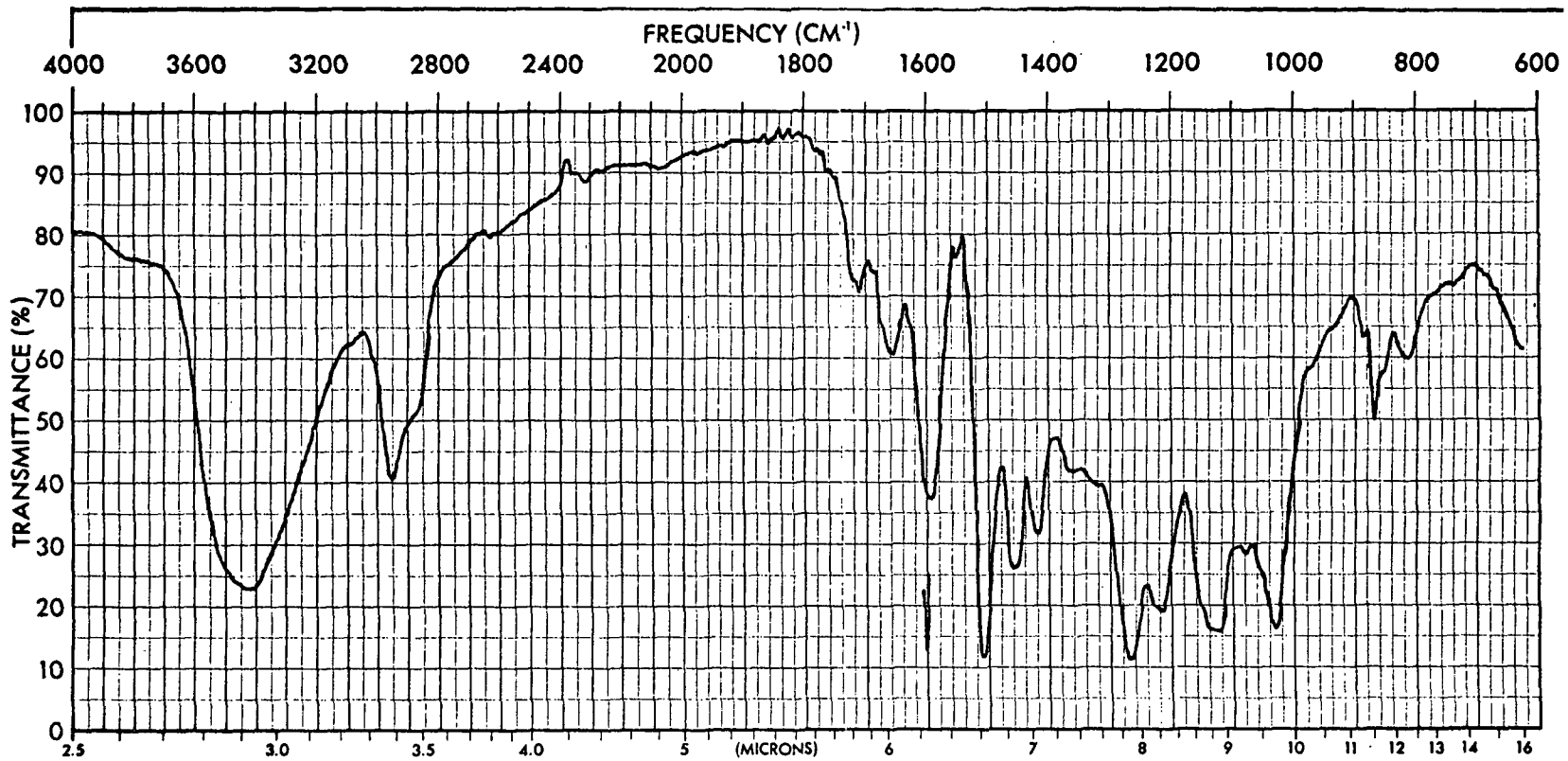


Figure 10. Infrared Spectrum of Loblolly Pine Dioxane Lignin

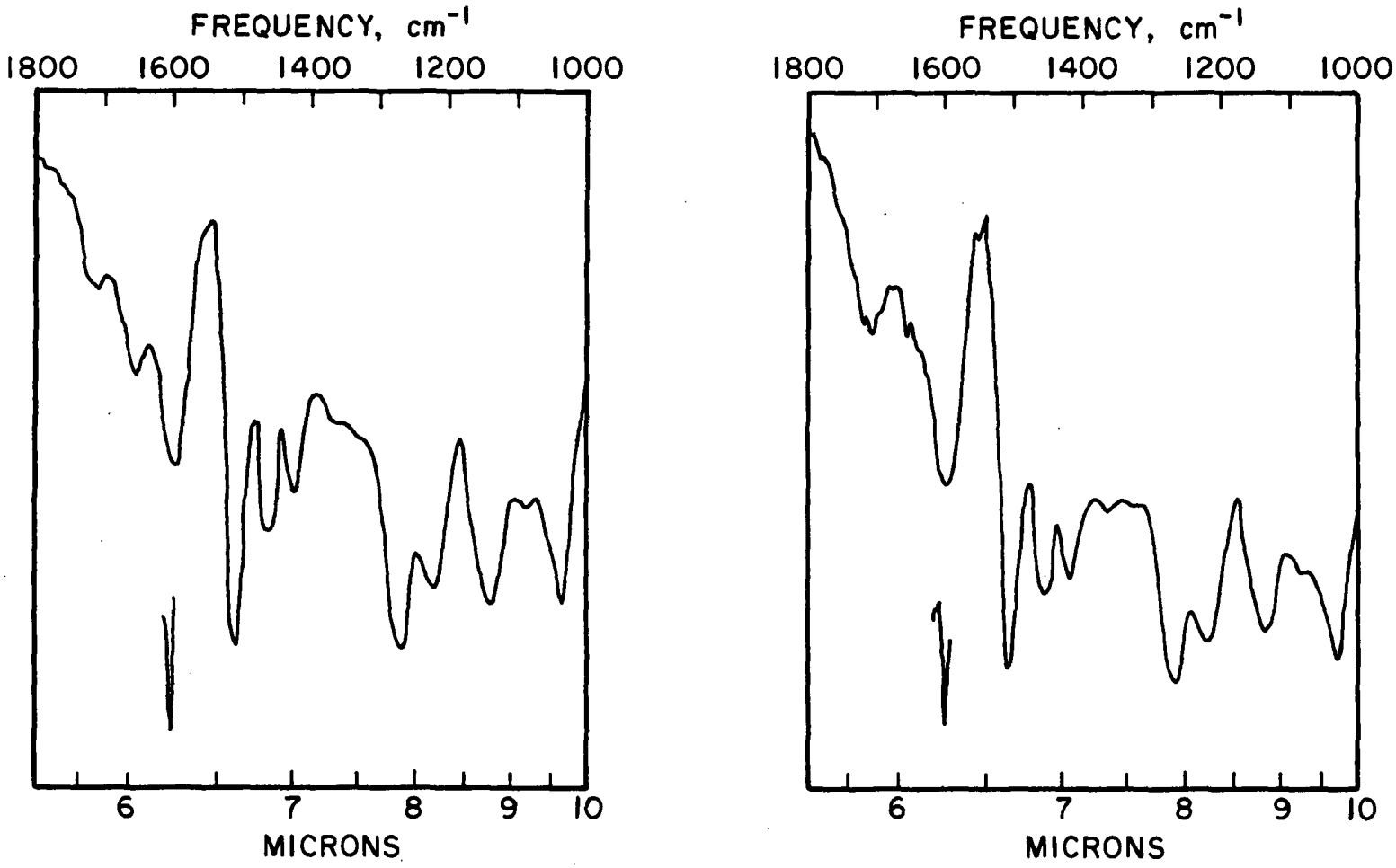


Figure 11. Infrared Spectra of Loblolly Pine Dioxane Lignin

The spectrum on the left is the original material. In the spectrum on the right, the dioxane lignin has been reduced with sodium borohydride.

while the 1715 cm^{-1} band appeared to be unaffected (Fig. 11), indicating that the 1715 cm^{-1} band cannot be assigned to a β-ketone. The C=O group of dimerized saturated aliphatic carboxylic acids also absorbs in the region of 1720-1706 cm^{-1} and near 1400 cm^{-1} (57). Comparison of the IR spectra of DL samples freeze dried at pH 3 and at pH 9 failed to show any difference in the 1715 cm^{-1} band intensity, indicating it was not related to the carboxylic acid group. It thus appears that the 1715 cm^{-1} band may arise from aryl esters which absorb in the region of 1730-1715 cm^{-1}. The C-O stretching vibrations (asymmetric) for aryl esters are found in the region 1310-1250 cm^{-1}, where there is already strong absorbance which may mask these bands (57). The presence of aryl ester groups in the DL would not be expected to significantly affect the reactivity of the lignin toward degradation with oxygen in alkaline medium.

CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Carbon-13 (^{13}C) NMR is particularly useful for the study of lignin structure which cannot be investigated by other analytical procedures. Ludemann and Nimz (72,73) studied ^{13}C NMR spectra of MWL isolated from spruce and identified 31 carbon atoms in the lignin by comparing the ^{13}C shifts to those of 14 monomeric and 25 dimeric lignin model compounds.

The proton decoupled ^{13}C NMR spectrum of the DL is compared with the spectrum of spruce MWL (inset, Ref. 73) in Fig. 12. Peak assignments are summarized in Table VI, with the model compounds (numbers in parentheses) from which the assignments were made included in Appendix III.

With the exception of signal number 30, all signals assigned by Ludemann and Nimz (73) in spruce MWL were identified in the DL spectrum. Agreement between shift values was excellent (Table VI). The signal to noise ratio of the spruce MWL spectrum was somewhat better than that obtained for the DL,

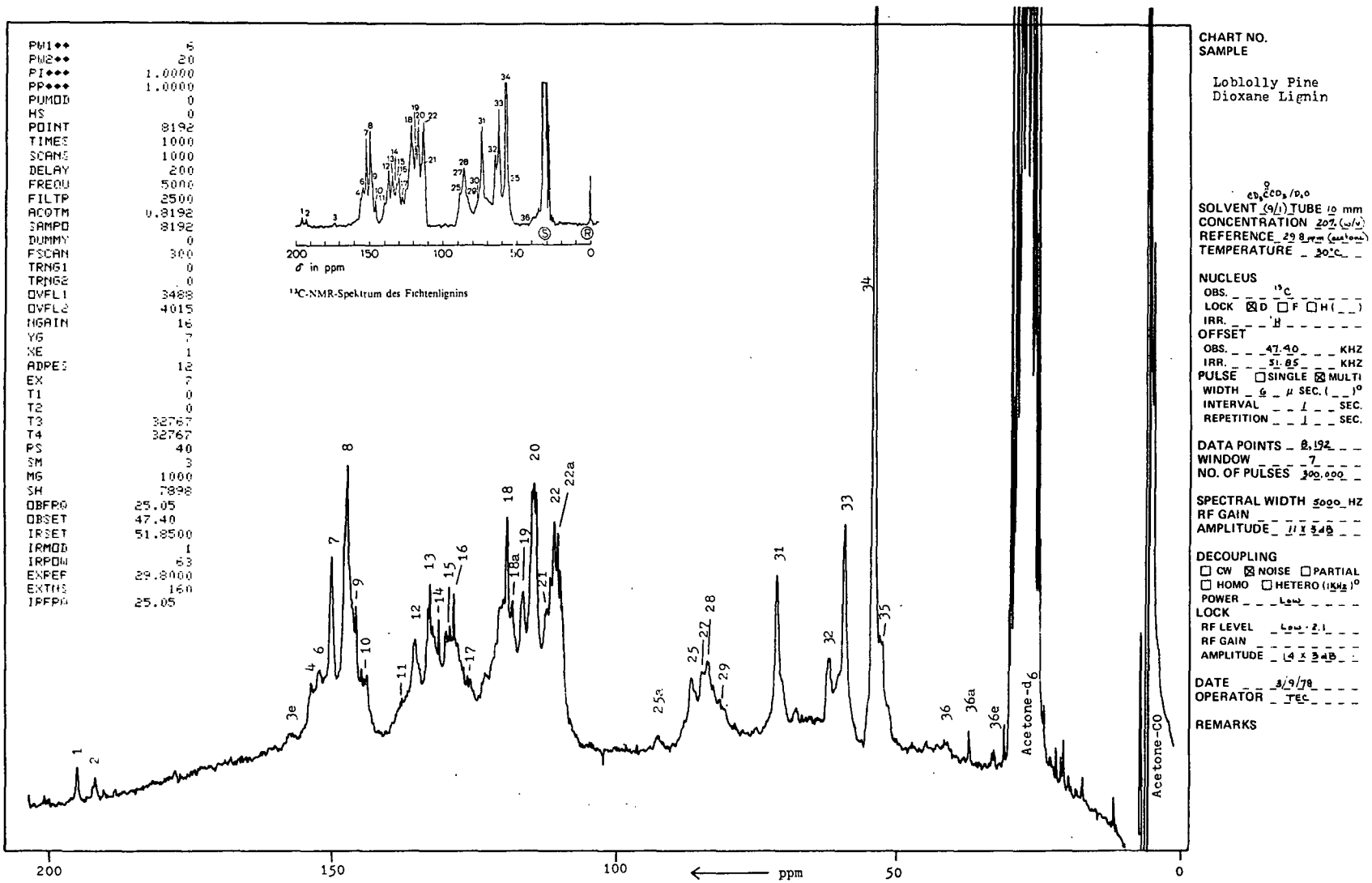


Figure 12. ^{13}C NMR Spectrum of Loblolly Pine Dioxane Lignin

but the resolution of signals in the DL spectrum was superior to that of the MWL spectrum. Several signals not observed in the MWL spectrum were noted in the DL spectrum and have been designated with letters following the number.

TABLE VI

ASSIGNMENTS OF THE CHEMICAL SHIFTS IN THE ^{13}C-NMR SPECTRUM OF SOFTWOOD LIGNIN (73)

Signal Number	δ-Value in ppm[a,b] Spruce MWL (73)	Loblolly Pine DL (this work)	Assignment[c]
1	195.2 w	194.6 w	γ-CO in cinnamaldehyde (1a) and α-CO (5c)
2	192.4 w	191.4 w	α-Aldehyde (2c, 3c-f, 5b, 5c, 6b)
3e	--	157.8 w	Not assigned
4	154.5 w	154.7 w	C-4 in guaiacyl with α-CO, etherified (5b, 5c)
6	152.9 vw	152.7 vw	C-4 in guaiacyl with α-CO (5a), with α-CHO, etherified (5b, 5c)
7	150.7 s	150.4 s	C-4 in guaiacyl, etherified (4a-d, 5a, 6a, 12d) and C-3 in guaiacyl with α-CO, etherified (5b, 5c, 6b)
8	148.1 s	147.7 s	C-3 in guaiacyl (2a, 2b, 2d, 2e, 4a-d, 5a, 8a, 10b, 10c, 11a, 11b, 12a, 12b)
9	146.9 m	146.1 m	C-4 in guaiacyl (2a, 2d, 4a, 4b, 4d, 10b, 10c, 12a), and C-α in cinnamic acids (1b, 2e, 3g)
10	144.8 w	144.8 w	C-α in cinnamic acids (11c) and C-4' in phenylcoumarans (10b)
11	138.8 w	138.7 w	C-1 in (12a)
12	136.2 m	136.2 m	C-1 in guaiacyl, etherified (4c)
13	133.6 m	133.3 m	C-1 in p-hydroxyphenyl and guaiacyl (3a, 4a, 4b, 4d, 7a, 7b, 10a, 10c)
14	132.7 vw	131.8 w	C-β in cinnamaldehyde (1a), C-1 in guaiacyl with α-CH_2-C- (8a)
15	130.2 w	130.3 m	C-1 with α-CH=C- or α-CH-sec (2d, 9) and C-1 in phenylcoumaran (10c)
16	129.0 m	129.2 m	C-2 and C-6 in p-hydroxyphenyl (7a, 10a) and C-β in cinnamalcohols (10a, 10c)
17	126.7 w	126.8 w	C-6 in guaiacyl with α-CO, etherified (5b, 5c)
18	120.2 s	120.0 s	C-6 in guaiacyl (4a-d, 10b, 10c)

TABLE VI (Continued)

ASSIGNMENTS OF THE CHEMICAL SHIFTS IN THE ^{13}C-NMR SPECTRUM OF SOFTWOOD LIGNIN (73)

Signal Number	δ-Value in ppm[a,b] Spruce MWL (73)	Loblolly Pine DL (this work)	Assignment[c]
18a	--	119.1 m	Not assigned
19	117.4 m	117.4 m	C-6' in phenylcoumaran (10b)
20	115.7 s	115.4 s	C-5 in guaiacyl (2a-d, 4a, 4b, 4d, 10b, 10c) and C-3/C-5 in p-hydroxyphenyl (7a, 10a)
21	113.8 w	113.0 w	C-2 in guaiacyl with α-CO or α-CH_2- (4d, 5a-c, 8a)
22	112.1 s	112.0 s	C-2 in guaiacyl (4b-d)
22a	--	111.2 s	Not assigned
25a	--	93.8 w	Not assigned
25	88.1 w	88.0 w	C-α in phenylcoumaran (10)
27	86.1 m	85.9 m	C-β in phenylglycerin-β-aryl ethers (4d)
28	85.2 m	85.0 m	C-β in β-aryl ethers with an α-CO (5c)
29	81-83 w	82.9 w	C-β in β-aryl ethers with an α-CO
30	75.0 w	--	C-α in β-1-dilignols (9)
31	73.1 s	73.0 s	C-α in β-aryl ethers (4d), C-γ in dibenzyltetrahydrofuran units (8) and in pinoresinol units (7)
32	63.8 m	64.1 m	C-γ and C-β in β-1-dilignols (9), C-γ in β-aryl ethers with an α-CO (5c), and C-γ in phenylcoumaran (10) and cinnamalcohols (10a, 10c)
33	61.4 s	61.2 s	C-γ in β-aryl ethers (4d)
34	56.2 vs	56.2 vs	OCH_3
35	54.8 w	54.6 w	C-β in pinoresinol units (7) and phenylcoumaran (10)
36	40-42 vw	42-45 vw	C-α in dibenzyltetrahydrofuran (8)
36a	--	39.4 w	Not assigned
36e	--	35.1 vw	Not assigned

[a] δ-Values in ppm from TMS; spectra run in deuteroacetone/deuterium oxide (9/1, v/v)

[b] Intensities: w = weak, m = medium, s = strong, vw = very weak, vs = very strong.

[c] Numbers in parentheses refer to model compounds (Appendix III) from which the assignments were made.

There were some differences in signal intensities between the two spectra. In the DL spectrum signal 8 was significantly greater than signal 7, but in the MWL spectrum, these signals had approximately the same intensity. Also, signal 20 in the DL spectrum was more intense than signals 18 and 22, but in the MWL spectrum these signals had comparable intensities.

The difference in intensities, as well as the additional signals observed in the DL spectrum, may represent differences in pulse times, species differences, or changes in the lignin structure resulting from the different isolation procedures. As has been mentioned previously, model compound studies (39) have indicated that a major consequence of the isolation of DL with 0.2N HCl is the cleavage of benzyl ether linkages in the lignin. Based on infrared spectra it has been suggested that some β-aryl ether linkages may also be cleaved in the isolation of DL (69). However, the region of the ^{13}C NMR spectrum which would be expected to show the greatest response to these types of reactions occurs between signals 25 and 35 (C-α, C-β, C-γ). There was no apparent difference in this region between the DL and MWL spectra.

PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Proton nuclear magnetic resonance (PMR) spectroscopy possesses several unique features which make it an attractive analytical tool for the study of organic materials. Two of these features are of particular interest in the study of lignin systems. First, PMR spectroscopy is able to differentiate between protons in different chemical environments in the molecule and second, PMR spectroscopy is able to relate the area of the absorption signals to the quantitative determination of the relative amount of each type of proton detected.

Unfortunately, PMR loses much of its effectiveness when applied to high molecular weight materials such as isolated lignins and their derivatives because of the large variety of different chemical environments in the polymer. The PMR spectra of isolated lignins show broad, ill-defined bands which represent many overlapping individual signals. In spite of these limitations, PMR spectroscopy has been applied to the study of lignin model compounds and derivatized lignin preparations by a number of investigators (74-80). Most of these studies have used acetylated lignin derivatives with deuterated chloroform as solvent. The DL used in this investigation was likewise acetylated and studied using deuterated chloroform as solvent.

Chemical shifts for acetylated lignin model compounds have been used to assign regions of the PMR spectrum to characteristic proton environments. PMR studies of isolated lignins and lignin model compounds have been reviewed in detail by Ludwig (81). Assignments used in this investigation are based on the work of Morohoshi and Sakakibara (79) (Table VII).

TABLE VII

RANGES OF CHEMICAL SHIFTS FOR PROTONS IN ACETYLATED LIGNINS (79)

Range	Symbol	δ-Values[a], ppm	Proton Types
1	H_a	8.00-6.28	Aromatic and α-vinylic
2	H_α	6.28-5.18	β-Vinylic and benzylic
3	H_{OCH_3}	5.18-3.15	Methoxyl
	$H_{\beta\gamma}$		β- and γ-, oxygen on same C
4	$H_{\alpha'}$	3.15-2.40	α-, without oxygen on α-C
5	H_{PhOAc}	2.40-2.19	Aromatic acetoxyl
6	H_{AlOAc}	2.19-1.52	Aliphatic acetoxyl
7	$H_{\beta'\gamma'}$	1.52-0.00	Highly shielded aliphatic

[a] δ-Values in ppm from TMS; spectra run in $CDCl_3$.

The PMR spectrum of the DL used in the present investigation is presented in Fig. 13. Acetylation was carried out with pyridine-acetic anhydride. In addition, a small amount of dimethylformamide (DMF) was added to aid in solubilization of the lignin and thereby promote acetylation (82). Subsequent washing of the acetylated product with acid and water did not remove all of the DMF. However, the DMF signals at δ = 2.88, 2.95, and 8.02 ppm were very sharp and in regions of the spectrum where the integral could be readily adjusted. The water peak at δ = 1.26 ppm and the chloroform peak at δ = 7.26 ppm were introduced with the deuterated chloroform and the integral was also corrected for these signals.

Semiquantitative interpretation of the PMR spectrum is made by relating the total integral of the spectrum to the calculated total protons per C_9 unit. The percent of total integral which appears within each range may then be related to the number of protons per C_9 signaling in each range. This procedure is based on the assumption that isolated lignins are totally composed of guaiacylpropane units. For the DL starting material this assumption may be expected to be fairly good. However, in the oxidized lignins discussed later in this thesis, this assumption could no longer be applied.

The integral of the PMR spectrum of the DL is presented in Table VIII and related to the total protons per C_9 unit calculated from the carbon-hydrogen and methoxyl analyses (Table II). The acetoxyl content of the integral has been adjusted (by dividing by 3) to the nonacetylated DL in order to be able to relate total protons in the PMR spectrum to the empirical C_9 formula calculated from the fundamental analyses (empirical formula: $C_9H_{7.81}O_{2.67}(OCH_3)_{0.97}$).

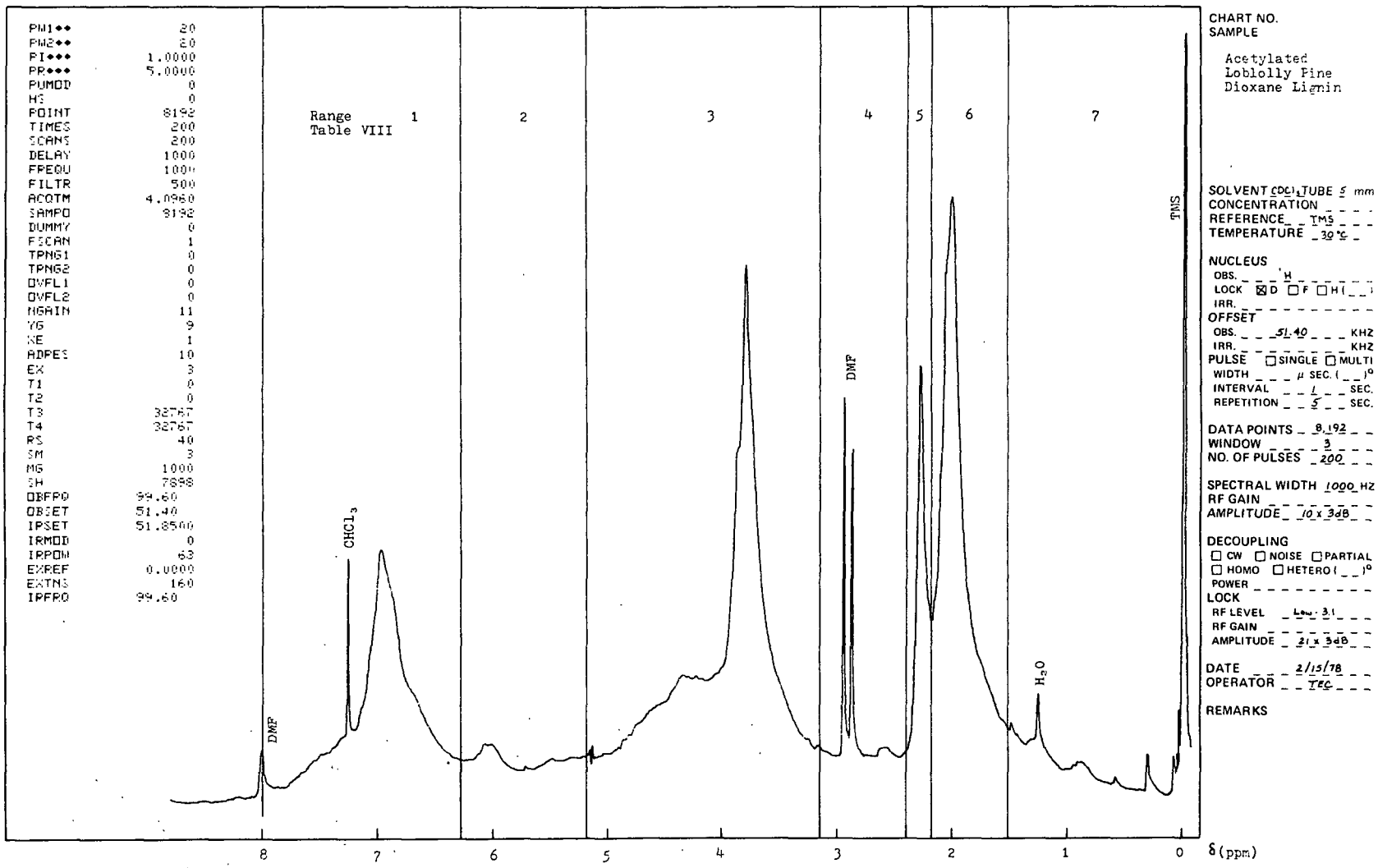


Figure 13. PMR Spectrum of Loblolly Pine Dioxane Lignin

TABLE VIII

HYDROGEN BALANCE IN LOBLOLLY PINE DIOXANE LIGNIN BASED ON FUNDAMENTAL ANALYSES AND PMR

Range[a]	Symbol	% of Total Integral	Protons per C_9	
			Present Work	Spruce MWL (78)
1	H_a	21.9	2.35	2.57
2	H_α	6.5	0.70	0.66
3	H_{OCH_3}	25.7	2.76	2.55
	$H_{\beta\gamma}$	24.2	2.60	
4	$H_{\alpha'}$	4.9	0.53	3.26
5	H_{PhOH}	3.2	0.34	0.28
6	H_{AlOH}	9.1	0.98	0.93
7	$H_{\beta'\gamma'}$	4.6	0.49	0.06

[a]Ranges and assignments summarized in Table VII.

Results for the hydrogen balance presented in Table VIII for the DL were compared with values calculated by Lenz (78) for spruce MWL. With the exception of the $H_{\beta'\gamma'}$ range, there was generally good agreement between the present work and that of Lenz (78). The very high proton level calculated for the $H_{\beta'\gamma'}$ range in the DL was thought to arise from a baseline problem in this region of the NMR spectrum.

The range in which aromatic protons signal, δ = 6.28 to 8.00 ppm, has been reported to be relatively free from other types of protons (81). Thus it has been possible to relate the integral in this region, with some confidence, to the number of aromatic protons per C_9 unit. This value may in turn be used to estimate the number of "condensed" and "noncondensed" aromatic nuclei in the DL, where a condensed nucleus is defined as one containing two protons attached to the aromatic ring. Examples of condensed linkages which have been identified in lignin include β-O-4, β-5, β-1, 5-5, 4-O-5,

and 1-O-4 structures. A noncondensed nucleus is defined as one having three protons. Based on this reasoning, the DL contained 65% condensed units. Ludwig (81) reported that spruce MWL typically contains only about 40-50% condensed units, while lignin isolated under acidic conditions, such as the DL used in this study, typically contains 70-80% condensed units.

Phenolic hydroxyl contents have been estimated from PMR spectra of acetylated lignins (75,79). The phenolic hydroxyl content of the DL is discussed in the following section.

PHENOLIC HYDROXYL

The importance of the phenolic hydroxyl group in proposed mechanisms of lignin degradation with O/A has been discussed in the Introduction. No single method for the determination of free phenolic hydroxyl groups in lignin has gained universal acceptance. Two methods which have been used by several investigators are based on UV difference spectroscopy and PMR spectroscopy. Because of the importance of phenolic hydroxyl groups in the present investigation, both of these methods were applied to the determination of the phenolic hydroxyl content of the DL.

Phenolic hydroxyl content of the DL was determined from the UV difference spectrum (Fig. 9) by the method of Wexler (83) as modified by Arseneau and Pepper (37). Phenolic hydroxyl content of the DL, calculated from the UV difference spectrum, was 2.4% (0.27 PhOH/C_9 unit). This was in good agreement with a value of 2.3% phenolic hydroxyl reported by Arseneau and Pepper (37) for spruce DL. Chang, et al. (34) determined phenolic hydroxyl from UV difference spectra and reported contents of 0.202 and 0.190 PhOH/C_9 unit for spruce MWL and CEL, respectively.

Phenolic hydroxyl content of the DL was also determined from the PMR spectrum (Fig. 13, Table VIII) and from the carbon-hydrogen analysis (Table II) according to the procedure of Morohoshi and Sakakibara (79). Phenolic hydroxyl content, calculated based on the integral of the PMR spectrum, was 3.1% (0.34 PhOH/C_9 unit). Morohoshi and Sakakibara (79) reported a phenolic hydroxyl content of 0.37 PhOH/C_9 unit in spruce MWL by the PMR technique. Ludwig, et al. (75) found 0.35 PhOH/C_9 unit in western hemlock DL and 0.28 PhOH/C_9 unit in spruce MWL by the PMR technique.

The higher phenolic hydroxyl content calculated by the PMR technique, relative to the UV difference method, has been noted previously (81). The reason for the difference is not understood. It has been suggested that the high values characteristically calculated from PMR spectra of acetylated lignins may result from shielding effects or from an unrecognized type of proton which signals in the phenolic acetoxyl region of the spectrum (81).

CARBONYL

Adler and Wallden (84) reported a carbonyl content of 0.19 CO/C_9 unit in spruce MWL using an oximation procedure. By reduction and methylation techniques Adler and Marton (85) assigned the carbonyl groups in spruce MWL to 0.03 cinnamaldehyde unit/C_9 unit, 0.07 α-CO/C_9 unit and, by difference, 0.09 β-CO/C_9 unit. Carbonyl content of the DL used in this study was 0.18 CO/C_9 unit, determined by oximation (86).

CARBOXYL

The carboxyl content of the DL was estimated to be 0.02 COOH/C_9 unit by potentiometric titration. Ishikawa, et al. (24) reported a carboxyl content

of 0.10 COOH/C_9 unit in pine DL. Adler, et al. (87) reported a carboxyl content of 0.05 COOH/C_9 unit in spruce MWL.

MOLECULAR WEIGHT DISTRIBUTION

The molecular weight distribution of the DL was determined according to the gel permeation chromatography (GPC) technique. Basic principles of GPC have recently been reviewed by Anderson (88) and by Snyder and Kirkland (89).

GPC is a form of liquid partition chromatography in which the solute molecules are distributed between the solvent in the gel pores and the solvent in the interstitial volume between the gel beads. Separation of solute molecules is achieved based on the size in solution of the molecules.

The most favored mechanism for GPC separation is the steric exclusion mechanism proposed by Laurent and Killander (90). According to this mechanism, it is assumed that different fractions of the total pore volume are accessible to molecules of different size, because the gel particles contain a distribution of pore sizes. Molecules which are too large to enter the pore matrix of the gel are said to be totally excluded and pass directly down through the column in the interstitial regions. Molecules of intermediate size can enter some of the pores and are thereby retarded. The smallest molecules enter a relatively larger number of pores, are most extensively retarded, and are eluted from the column last.

Molecular weight separation by GPC may be viewed in terms of a distribution coefficient, K_d. For a packed column of total volume, V_t:

$$V_t = V_m + V_s + V_p$$

where V_m = the interstitial volume, excluded volume, or void volume

V_s = volume of solvent in the pores of the gel

V_p = volume occupied by the porous gel particles

The elution volume, V_r, is defined as that volume of solute eluted from the column from the moment that the sample is introduced to the top of the column until the solute begins to appear in the eluate. The distribution coefficient, K_d, may then be defined by:

$$K_d = \left(V_r - V_m\right)/V_s$$

Thus, a graph of molecular size versus elution volume will have a linear range of permeation between the limiting values $K_d = 0$, corresponding to total exclusion and $K_d = 1$, corresponding to total permeation.

The molecular weight distribution of the DL was determined with Sephadex G-50 using formamide as solvent according to the procedure of Brown, et al. (91) (Fig. 14). Formamide was found by these authors to have negligible differential solvation and gel interaction effects. However, in aqueous solvents, these effects had a pronounced role in separation.

Void volume of the column was 130 ml. Two low molecular weight lignin model compounds were eluted from the column to calibrate the low molecular weight region: vanillic acid (MW = 168) and 1-(3,4-dimethoxyphenyl)-2-(2-methoxy-4-methylphenoxy)-1-propanone (MW = 330).

The inset in Fig. 14 shows the molecular weight distribution for spruce MWL obtained by Kirk and Chang (92) on a column of Sephadex G-50 using formamide as solvent. The general shape of the molecular weight distribution curves of the DL and spruce MWL were similar. The highest molecular weight portion of both lignins was too large for resolution by the gel.

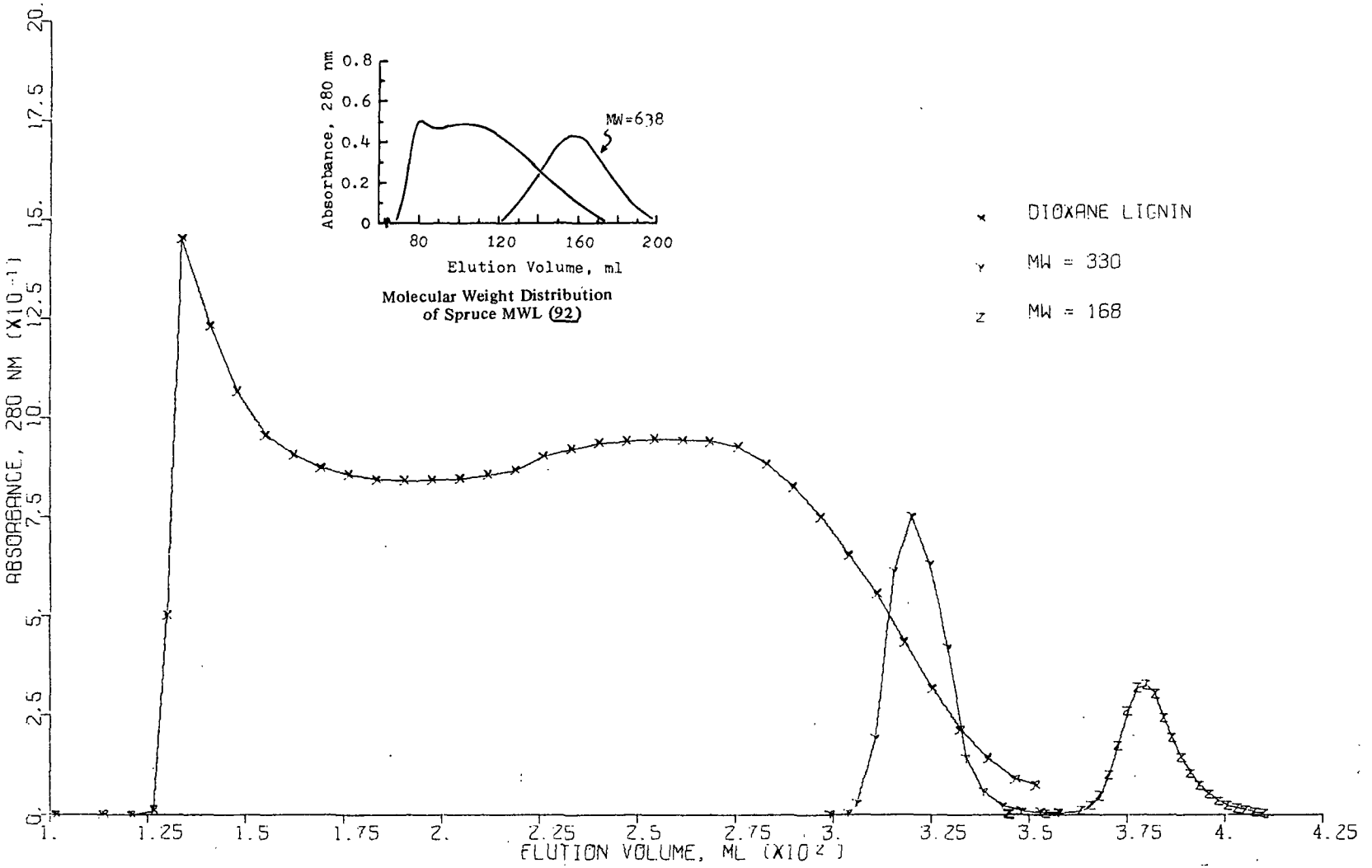


Figure 14. Molecular Weight Distribution of Loblolly Pine Dioxane Lignin Indicated by Chromatography on a Column of Sephadex G-50, with Formamide as Solvent. Two Lignin-Related Model Compounds with MW = 168 and 330 are included

Weight average molecular weight ($\bar{M}_w$) of the DL was estimated to be 11,000, determined by the conventional equilibrium sedimentation method on an analytical ultracentrifuge. The method of molecular weight calculation by equilibrium sedimentation is reviewed in detail in Appendix IV. Bjorkman and Person (28) likewise reported a value of 11,000 for the $\bar{M}_w$ of spruce MWL. Arseneau and Pepper (37) obtained a value of 15,000 for the $\bar{M}_w$ of spruce DL. Kirk and Chang (92) also obtained a value of 15,000 for the $\bar{M}_w$ of spruce MWL. The differences in $\bar{M}_w$ reported by the various workers cited may reflect the difficulty in obtaining an accurate value for $\bar{M}_w$, rather than real differences.

SUMMARY — ISOLATION AND CHARACTERIZATION OF DIOXANE LIGNIN STARTING MATERIAL

Sufficient DL for the investigation was isolated from loblolly pine (Pinus taeda L.) according to a procedure modified from Pepper, et al. (35,36). The lignin was homogenized and extensively characterized.

Comparison of analytical results with literature values for other mildly isolated softwood lignins led to the following conclusions:

1. Carbohydrate content (1.4%) was somewhat lower than levels normally reported for mildly isolated lignins.
2. Ash content (3.1%) was significantly higher than levels normally reported for mildly isolated lignins.
3. Carbon-hydrogen and methoxyl analyses were consistent with published values for mildly isolated lignins.
4. Ultraviolet spectra were consistent with published spectra for mildly isolated lignins.
5. The infrared spectrum of the DL contained a band at 1715 cm^{-1} which was more intense than is normally seen in MWL and CEL

preparations. This band has been assigned to β-ketones formed during the isolation procedure (71). However, reduction of the DL with sodium borohydride did not affect the intensity of the band. It is proposed that the band may be assigned to aryl esters in the DL.

6. The ^{13}C NMR spectrum of the DL was similar to the ^{13}C NMR spectrum of spruce MWL, differing principally in the intensities of some signals.

7. Integration of the PMR spectrum of the acetylated DL indicated that the DL was similar to spruce MWL. Analysis of the aromatic proton region suggested that there was some condensation of the aromatic rings in the DL as a result of the isolation procedure.

8. The phenolic hydroxyl content computed from the UV difference spectrum (2.4%) was somewhat higher than values reported by the same method for spruce MWL and CEL. However, the phenolic hydroxyl content calculated from the integral of the PMR spectrum of the acetylated DL (3.1%) appeared to be consistent with values reported for mildly isolated softwood lignins, determined by PMR spectroscopy.

9. Carboxyl and carbonyl contents of the DL were consistent with literature values for mildly isolated softwood lignins.

10. The molecular weight distribution of the DL was similar to the molecular weight distribution reported for spruce MWL. The weight average molecular weight (11,000) was identical to that reported for spruce MWL (28) by one group, but lower than values reported for other mildly isolated lignins (15,000, 37,92).

Based on chemical characterization, with the exception of structural modifications noted above, the DL isolated for this investigation appeared to be representative of mildly isolated softwood lignins.

OXIDATION OF DIOXANE LIGNIN WITH OXYGEN/ALKALI — NOMENCLATURE AND GENERAL PROCEDURES

Three series of lignin oxidations were made to investigate changes in the chemical structure of the lignin as a function of time of oxidation. In the following section the role of alkali concentration is evaluated by comparison of two oxidation series carried out at different alkali concentrations. Sodium carbonate was used as the alkali in all runs. Samples oxidized in the presence of 0.13N Na_2CO_3 are designated as OX.13, while samples oxidized in the presence of 0.50N Na_2CO_3 are designated as OX.50. The oxidation heading is followed by the length of time, in hours, that the sample was reacted. Thus, sample OX.50-4.0 was oxidized for 4.0 hours in 0.50N Na_2CO_3.

A modified lignin was prepared by pretreatment of the DL with peroxyacetic acid (PAA) (0.50 mole PAA/equivalent weight of lignin) (DL formula weight = 188.9). The PAA-modified lignins are designated as OX-PA-time. The modified lignin was oxidized in the presence of 0.13N Na_2CO_3 only. Analyses of the OX-PA samples are compared with those of the OX.13 samples in the subsequent section.

Lignin oxidations were carried out in a 150-ml Teflon-lined reactor fitted with an alkali injection device, an internal thermocouple, an oxygen charging line, and a gas sampling line. The reactor system is described in Appendix V. The following reaction conditions were used: 0.13N or 0.50N Na_2CO_3; 120°C; initial oxygen pressure = 0.618 MPa (89.7 psia, calculated at 120°C, Appendix VI); 1.0 g of lignin.

In a typical oxidation the lignin was added to the bottom of the reactor with 50 ml of triply distilled water. A weighed amount of alkali was placed in the Teflon injection tube and attached to the lid of the reactor on the oxygen charging line. The reactor and water were flushed with nitrogen to remove oxygen. The reactor was then sealed and brought to temperature in an oil bath.

The lignin slurry was stirred magnetically while the reactor was brought to temperature to prevent adhesion of the lignin to the surface of the Teflon liner. The glass transition temperature of the lignin was estimated to be 64°C by differential scanning calorimetry (Appendix VII). As was mentioned previously, the moisture content of the lignin was 5.6%. Goring (93) found that moisture lowered the glass transition temperature of isolated lignins. In preliminary experiments it was observed that the lignin, originally introduced as a fine powder, became tacky because the oxidation was carried out above the glass transition temperature. It was found that rapidly stirring the lignin/water slurry while the reactor was brought to temperature and the lignin/alkali solution during the oxidation prevented the lignin from adhering to the surface of the reactor.

Once the reactor was at temperature, oxygen was introduced for 1 minute at a pressure of 121 psig. The plug in the bottom of the injection tube was blown out by the oxygen and introduced the alkali to the reactor with the oxygen. The reaction solution was saturated with oxygen within 10 minutes after introduction of the oxygen (Appendix VIII).

The 0.0-hour sample was prepared by introducing the lignin and water to the reactor, bringing the reactor to temperature as described above, and charging with nitrogen at temperature to introduce the alkali to the

lignin slurry. The starting material sample (SM) was prepared by slurrying a 1.0-g sample of DL in a beaker with Na_2CO_3 (0.6841 or 2.6498 g) in 50 ml of triply distilled water. Comparison of the chemical properties of the 0.0-hour and SM samples gave a measure of the extent of lignin degradation which occurred in the process of bringing the reactor to temperature.

Reaction was terminated after the desired period of time by reducing the oxygen pressure to atmospheric and immersing the reactor in cold water. Two samples of the pressurized gas were collected in the OX.13 series and analyzed for carbon dioxide and carbon monoxide. Analyses of replicate gas samples were not reproducible. Gas analyses results and problems are discussed in Appendix IX.

The final reaction pH was measured and an estimate of unreacted base obtained by titration with 0.1N HCl to pH 7.0. The sample pH was next adjusted to a pH of 3.0 by addition of 1.0N HCl and the solution bubbled with nitrogen and stirred for 3 hours to remove CO_2. Finally, the pH was adjusted to pH 9.0 by addition of 1.0N NaOH and the sample freeze dried.

The C-9 unit structure (guaiacylpropane) of lignin used as a basis for calculation of various chemical properties of the DL in the previous section was not expected to hold for the oxidized samples discussed in the following sections. Therefore, all chemical analyses are reported on a percent basis [(weight of functional group/weight of lignin) x 100].

OXIDATION OF DIOXANE LIGNIN WITH OXYGEN/ALKALI — ROLE OF ALKALI CONCENTRATION ON CHANGES IN THE CHEMICAL PROPERTIES OF THE LIGNIN

Changes in the chemical properties of the DL as a function of time of oxidation are compared in this section for two oxidation series carried out

at concentrations of 0.13N and 0.50N Na_2CO_3. The OX.13 series was carried out first and it appeared that changes in a number of the chemical properties tended to level off after about 2 hours of reaction. A possible explanation for the leveling was that the pH of the oxidation liquor, which was approximately 9.4 after 2.0 hours, was no longer sufficiently alkaline to promote alkali-catalyzed degradation of the lignin. Therefore, an oxidation series was run at a higher initial alkali concentration in order to assure that the pH did not reach 9.4 during the 8 hours of oxidation.

FINAL pH AND BASE CONSUMPTION

The pH after oxidation and the milliequivalents of acid required to titrate each reacted sample liquor to pH 7.0 for oxidation runs OX.13 and OX.50 are given in Table IX. The final pH of the OX.50 series approached a pH of 9.4 only in the 8.0 hour sample. Therefore, comparison of lignin degradation in terms of functional group analyses might be expected to confirm the importance of maintaining the pH above 9.4. From a pulping point of view a fourfold increase in Na_2CO_3 concentration increased the initial pH by only 0.15 pH unit, still well below traditional pH levels found with sodium hydroxide which are associated with carbohydrate peeling reactions.

The number of milliequivalents of base consumed for a given time of oxidation was computed by subtracting the milliequivalents of acid to titrate to pH 7.0 for the given time of oxidation from the milliequivalents of acid required to titrate the SM sample. Base consumption was related qualitatively to production of acidic degradation products in the oxidation of the lignin. Base consumption as a function of time of oxidation is plotted in Fig. 15 for the two oxidation series. Both oxidation series were characterized by rapid initial base consumption. There was a good deal of scatter in the data from the titration of the OX.50 series, but it was

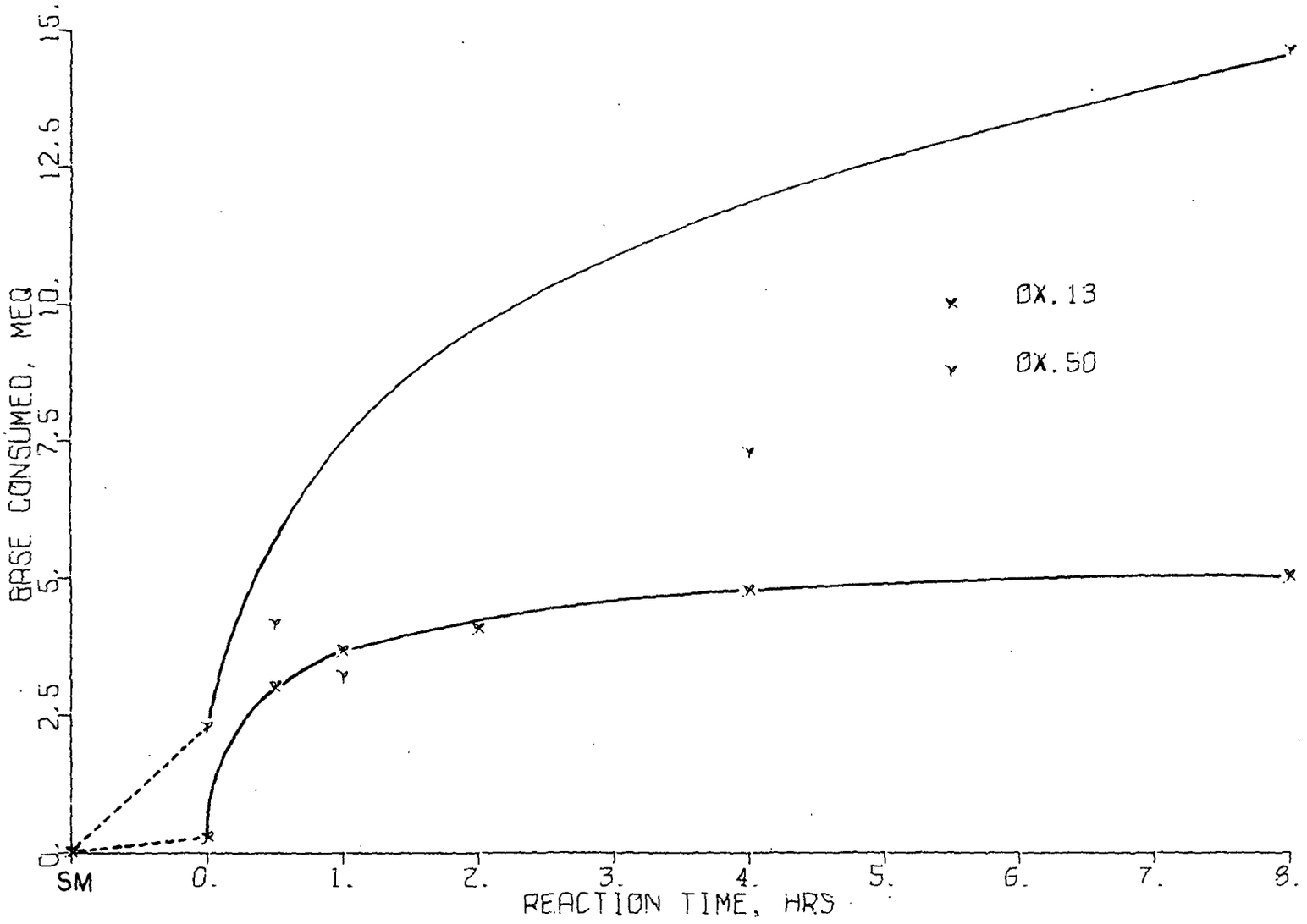


Figure 15. Base Consumption vs. Reaction Time for 0X.13 and 0X.50 Series

apparent that more alkali was consumed for a given time of reaction in the OX.50 run than in the OX.13 run. Whereas the OX.13 base consumption leveled off after about 2.0 hours, the OX.50 base consumption appeared to continue to increase throughout the full 8.0 hours of oxidation.

TABLE IX

pH AFTER REACTION AND MEQ ACID TO TITRATE TO pH 7.0

Sample Number	Oxidation Run OX.13		Oxidation Run OX.50	
	pH After Reaction	Meq Acid to Titrate to pH 7.0	pH After Reaction	Meq Acid to Titrate to pH 7.0
SM	10.965	7.51	11.128	30.35
0.0	10.970	7.24	11.155	28.07
0.5	10.079	4.50	10.639	26.20
1.0	9.669	3.83	10.485	27.16
2.0	9.486	3.44	9.912	20.07
4.0	9.433	2.74	9.738	23.07
8.0	9.322	2.47	9.538	15.75

ASH AND MOISTURE CONTENTS

Moisture contents, based on o.d. weight, are tabulated in Appendix I. Values of chemical analyses were corrected for moisture content for each oxidized lignin sample.

The ash contents of the OX.13 and OX.50 samples were computed by totalling the organic and inorganic weights added to each sample. The computed ash contents were used to correct the weights of the oxidized lignins to permit calculation of functional group analyses on a percent functional group in lignin basis. Two assumptions were made in applying the ash correction: first, that the material lost due to handling of the samples had the same percent ash content as the material recovered for analysis, and second, that the net change in the weight of the lignin due to loss of volatile organic degradation

products during freeze-drying and gain of oxygen on oxidation was negligible relative to the total recovered sample weight.

The validity of these assumptions was tested by determination of the sodium content of the recovered materials. Comparison of this value with the sodium added to the samples prior to freeze-drying gave the sodium yield, which could then be compared with the total recovered yield. The ash contents and comparisons of sodium and total yields are tabulated in Appendix X. Results indicated that the calculated ash was accurate to within 1 to 2% in most samples.

Ash contents (primarily NaCl) of the OX.13 and OX.50 samples were approximately 50 and 75%, respectively. No effort was made to reduce the ash content by dialysis, as it was felt this procedure would lead to extensive loss of low molecular weight degradation products.

METAL CONTENT

As was mentioned previously, trace levels of certain transition metals can have pronounced catalytic effects on the degradation of lignin with oxygen in alkaline medium. This investigation was concerned with looking at trends in changes in the chemical properties of the DL as a function of time of oxidation. By using the same amounts of lignin, sodium carbonate, and water in all runs for a given oxidation series, any catalysis which may have resulted from the presence of metal ions should have been the same for the entire run. Metal analyses of selected samples from the OX.13 series and the OX.50 series are presented in Table XXXII of Appendix II. No attempt was made to examine the effect of various metals on the rate of lignin degradation.

ELEMENTAL ANALYSIS AND METHOXYL CONTENT

Fundamental analytical data for the OX.13 and the OX.50 series are summarized in Table X. Methoxyl contents in both oxidation series decreased with increasing time of oxidation. Elemental analyses were adjusted for the elemental composition of the methoxyl contents in order to compensate for loss of methoxyl. The oxygen content increased with increasing time of oxidation. Increasing oxygen and decreasing methoxyl contents of the oxidized lignin would be consistent with formation of oxidized lignin degradation products such as carboxylic acids. Comparison of the OX.13 series data with the OX.50 series data indicated that, for a given time of oxidation, the lignin reacted at the higher alkali concentration was more extensively degraded.

TABLE X

METHOXYL AND ELEMENTAL ANALYSES FOR OX.13 AND OX.50 SAMPLES

Sample Number	Elemental Analysis[a,b] C, %	H, %	O, %	OCH_3, %	Elemental Analysis Adjusted for Methoxyl C, %	H, %	O, %
OX.13-SM	64.57	5.97	29.46	15.65	58.51	4.45	21.39
-0.0	59.15	6.21	34.64	14.83	53.41	4.77	26.99
-0.5	Not determined			13.47			
-1.0	59.36	5.40	35.24	13.07	54.30	4.13	28.50
-2.0	56.39	4.88	38.73	12.75	51.46	3.64	32.16
-4.0	51.51	4.34	44.15	11.31	47.13	3.24	38.32
-8.0	51.91	4.51	43.58	9.49	48.24	3.59	38.69
OX.50-SM	63.49	5.62	30.89	15.26	57.58	4.13	23.02
-0.0	65.01	6.22	28.77	14.42	59.43	4.81	21.34
-0.5	59.75	5.49	34.76	14.44	54.16	4.08	27.32
-1.0	62.28	5.34	32.38	13.32	57.12	4.04	25.51
-2.0	52.39	5.44	42.17	11.71	47.86	4.32	36.13
-4.0	52.48	3.79	43.73	8.22	49.30	3.00	39.49
-8.0	48.63	3.93	47.44	6.19	46.23	3.33	44.25

[a] Based on moisture-free (dried over P_2O_5 under vacuum) weight, corrected for ash.

[b] Average of duplicate determinations.

ULTRAVIOLET SPECTROSCOPY

Direct Ultraviolet Spectra

The direct UV spectra of the oxidized lignins from the OX.13 and OX.50 series are compared in Fig. 16 and 17, respectively. As the UV absorptivity is pH sensitive, all oxidized samples were adjusted to a pH of 9.0 prior to freeze-drying.

The OX.13 samples appeared to lack any consistent trend in terms of increasing or decreasing absorptivity at a given wavelength as a function of time of oxidation. The OX.50 samples on the other hand, with the exception of the 8.0-hour sample, appeared to have a constant absorptivity at 280 nm and increasing absorptivity at about 260 and 300 nm with increasing time of oxidation.

Analysis of the oxidized lignins indicated that methoxyl and phenolic hydroxyl (subsequent section) contents decreased with increasing time of oxidation. As both of these functionalities contribute to the absorption band at 280 nm, it would have been expected that, all other factors being the same, the absorptivity at 280 nm would have decreased with time of oxidation.

An increase in the absorptivity at the minima at about 260 and 300 nm was also reported by Kirk and Chang (92) who studied spruce MWL which had been oxidatively degraded by fungi. The increase at 260 nm was attributed to increased aromatic carboxyl content, while the increase at about 300 nm was assigned to an increased α-carbonyl content. An increased aromatic carboxyl content seems to be a likely consequence of the oxidation in the present work. However, carbonyl analyses indicated that carbonyl groups were rapidly consumed in the oxidation (discussed in a later section) and thus, the increased absorptivity at about 300 nm cannot be assigned to α-carbonyls. An alternative

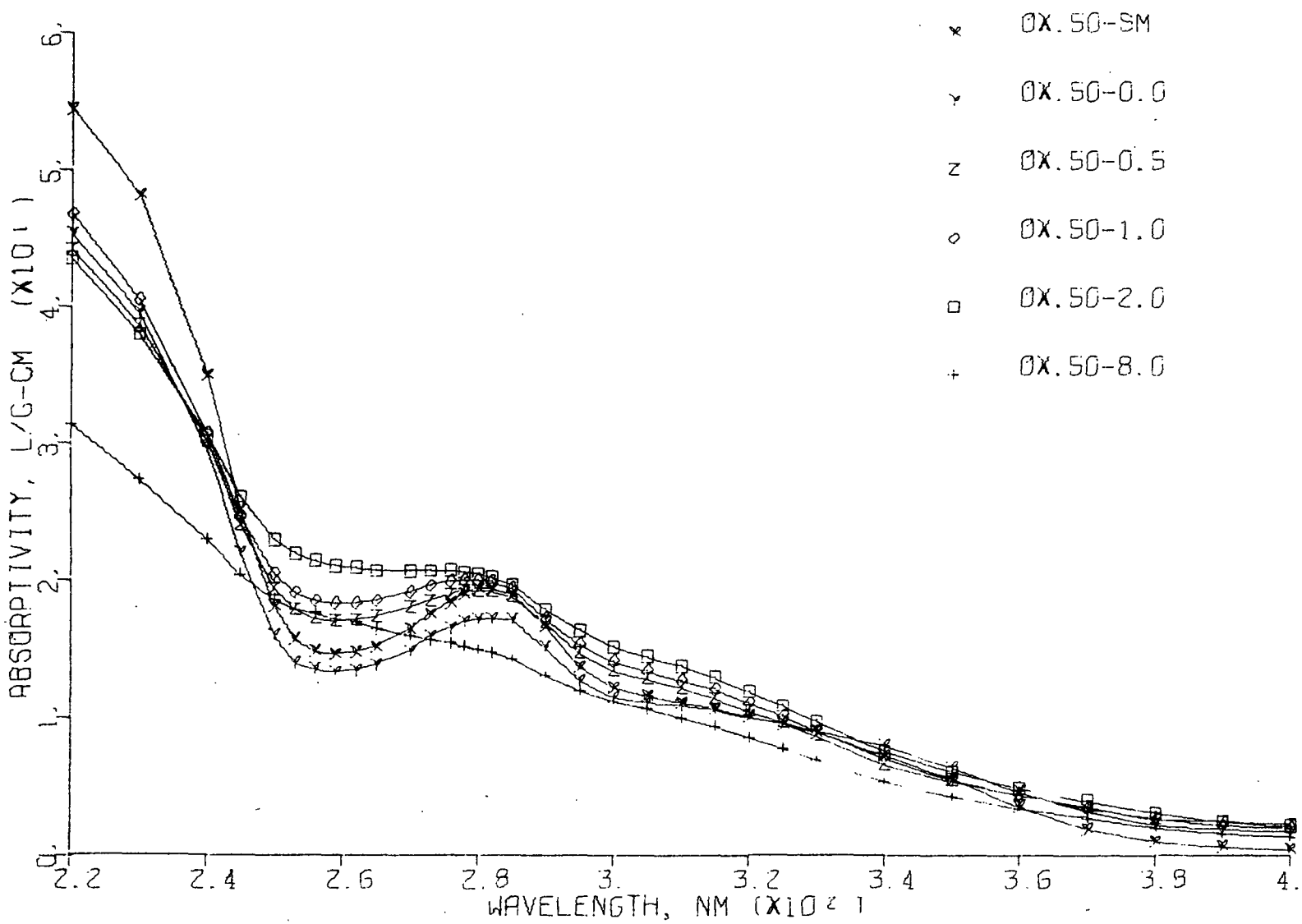


Figure 16. Comparison of Direct Ultraviolet Spectra of OX.13 Reaction Samples

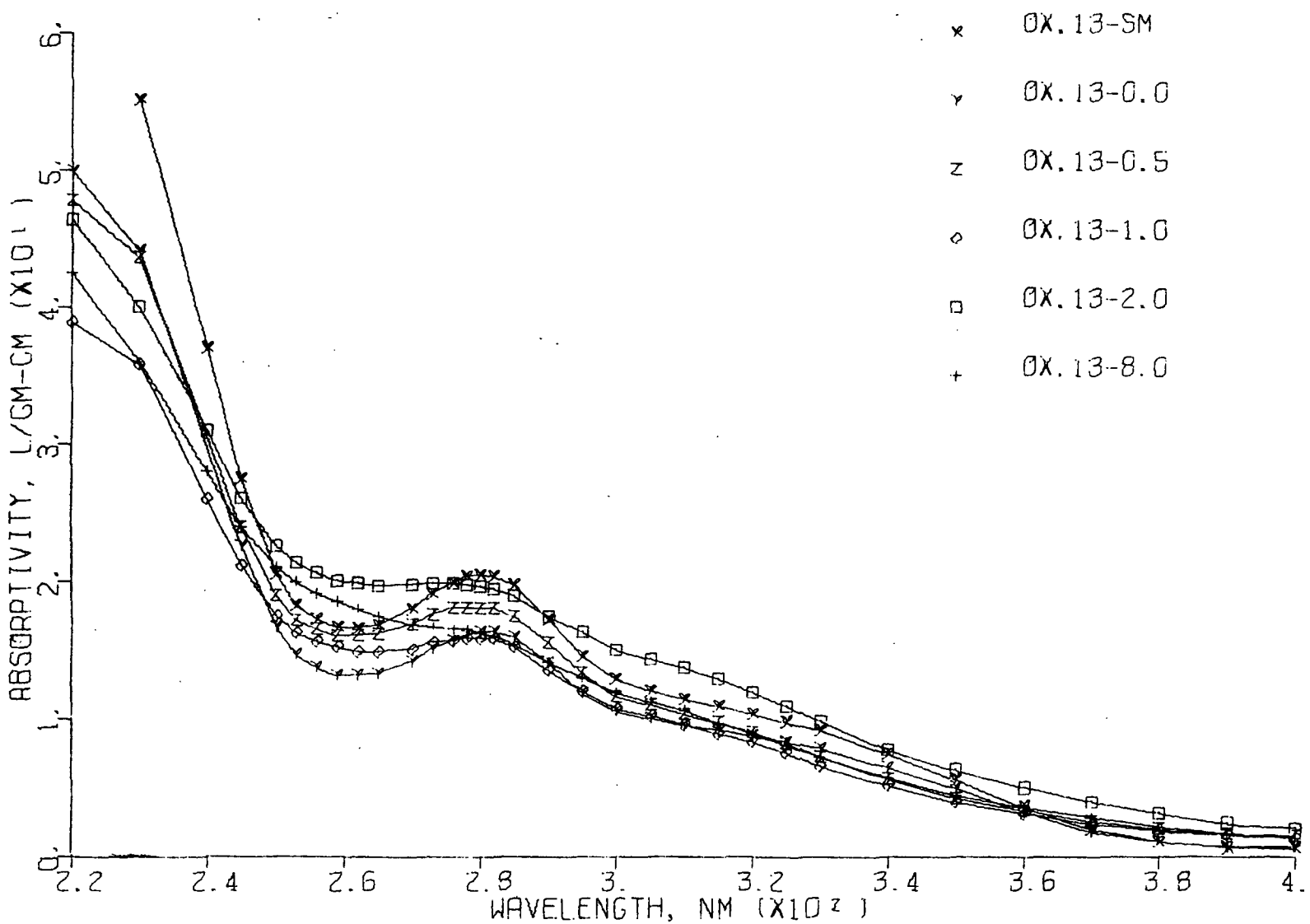
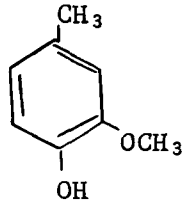


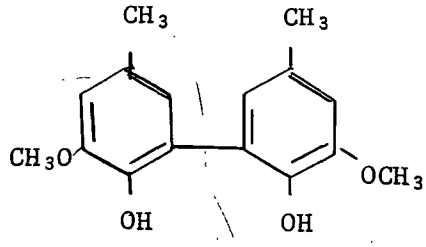
Figure 17. Comparison of Direct Ultraviolet Spectra of OX.50 Reaction Samples

conjugated π-system which absorbs at about 300 nm is the α-ethylenic group. Again, however, it seems unlikely that α-ethylenic groups would be formed in the oxidation of the lignin.

An alternative explanation which can be proposed to account for the increased absorbance at 260 and 300 nm and the lack of a significant decrease in the absorbance at 280 nm is the formation of biphenyl structures in the oxidation. Pew (58) reported that 5,5'-linked biphenyl structures related to lignin had a maximum absorbance at about 280 nm and high absorbance at 260 and 300 nm. Based on model compound studies, formation of biphenyl structures would be expected to be a reasonable consequence of the oxidation of the DL. Kratzl, et al. (11) reported that oxidation of creosol (III) with oxygen in alkaline medium led to extensive formation (60% of the starting material) of bicreosol (IV). The lower absorbance at 260, 280, and 300 nm



III



IV

observed for the OX.50-8.0 sample may be interpreted to indicate that the biphenyl structures were extensively degraded after 8.0 hours of oxidation.

The ready degradation of the biphenyl-linked structures is consistent with the work of Kratzl, et al. (11) in which they found that dihydroxybiphenyls were more easily oxidized than the corresponding monohydric phenol. This conclusion was based on the determination of the critical oxidation potential of these structures, which provides a measure of the ease of electron release from the phenol oxygen.

An attempt was made to evaluate the relative increase in absorbance at 280 and 300 nm in the two oxidation series by extending the flat portion of the curve between 300 and 320 nm to establish a baseline for measurement of the intensity of the band at 280 nm (Fig. 18). Results of this analysis are summarized in Table XI. The results in Table XI emphasize that, relative to any change in absorptivity between 300 and 320 nm, the absorptivity at 280 nm decreased with time of reaction, as would have been predicted, based on loss of methoxyl and phenolic hydroxyl groups. If one assumes that there was a linear relationship between absorptivity at 280 and at 300 nm due to formation of biphenyl structures, the above comparison lends support to the argument that the lack of a decrease in absorbance at 280 nm in the UV spectra of the oxidized lignins was due to formation of biphenyl structures.

TABLE XI

PEAK HEIGHTS AT 280 NM IN DIRECT ULTRAVIOLET SPECTRA

Sample Number	Oxidation Run OX.13 Absorptivity, 1/g-cm	Oxidation Run OX.50 Absorptivity, 1/g-cm
SM	4.91	5.16
0.0	3.92	4.28
0.5	3.85	2.90
1.0	2.67	2.80
2.0	1.63	2.06
4.0	1.06	1.47
8.0	1.25	1.15

The differences in absorptivity between the SM and 0.0-hour samples should also be noted. The decrease in absorptivity indicates that some attraction of the lignin resulted from bringing the reactor to temperature (93).

The direct UV spectra of the 2.0-hour and 8.0-hour samples from the two oxidation series are compared in Fig. 19 and 20, respectively. Within the

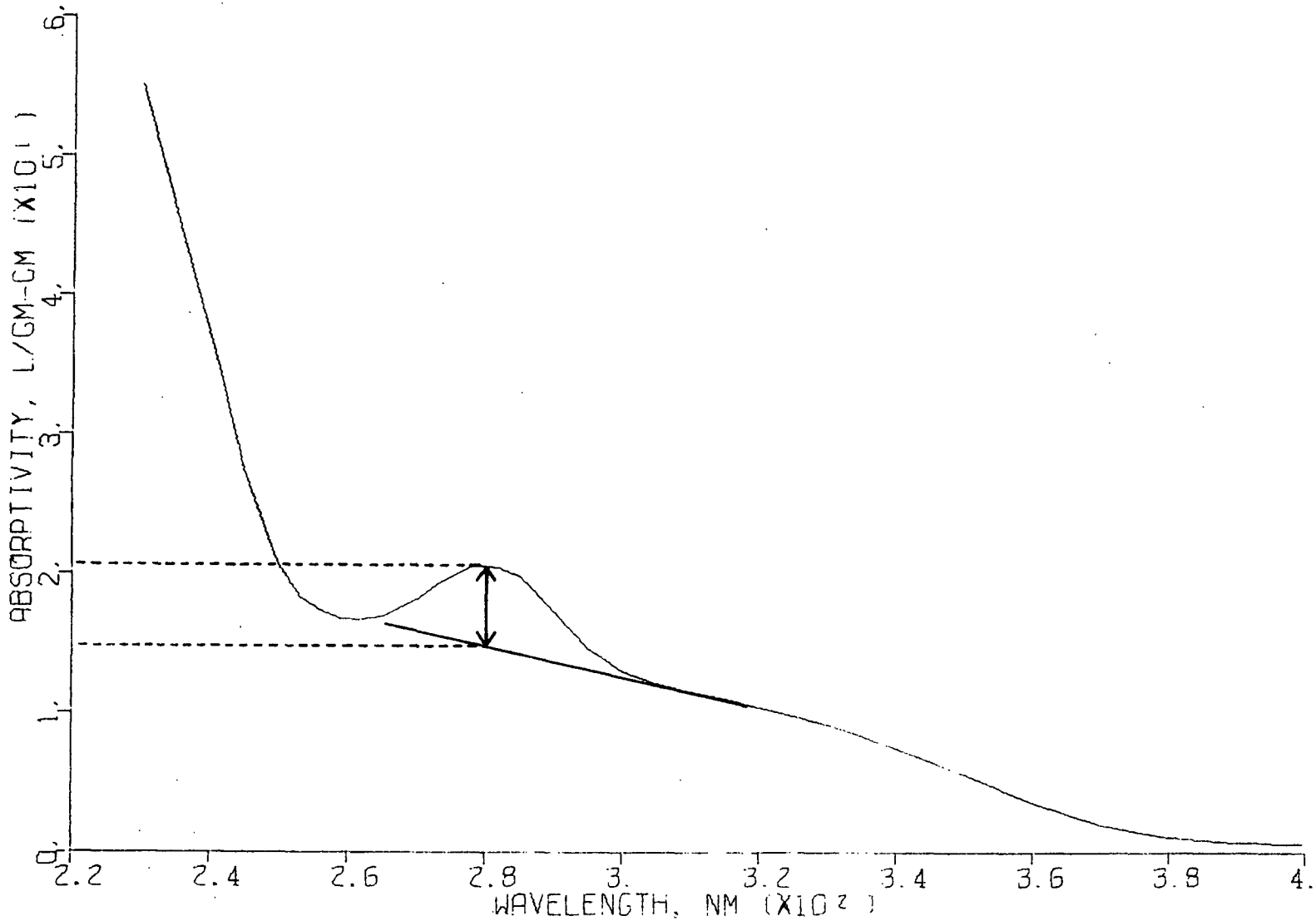


Figure 18. Method for Determination of Base Line for Calculation of Peak Height at 280 nm

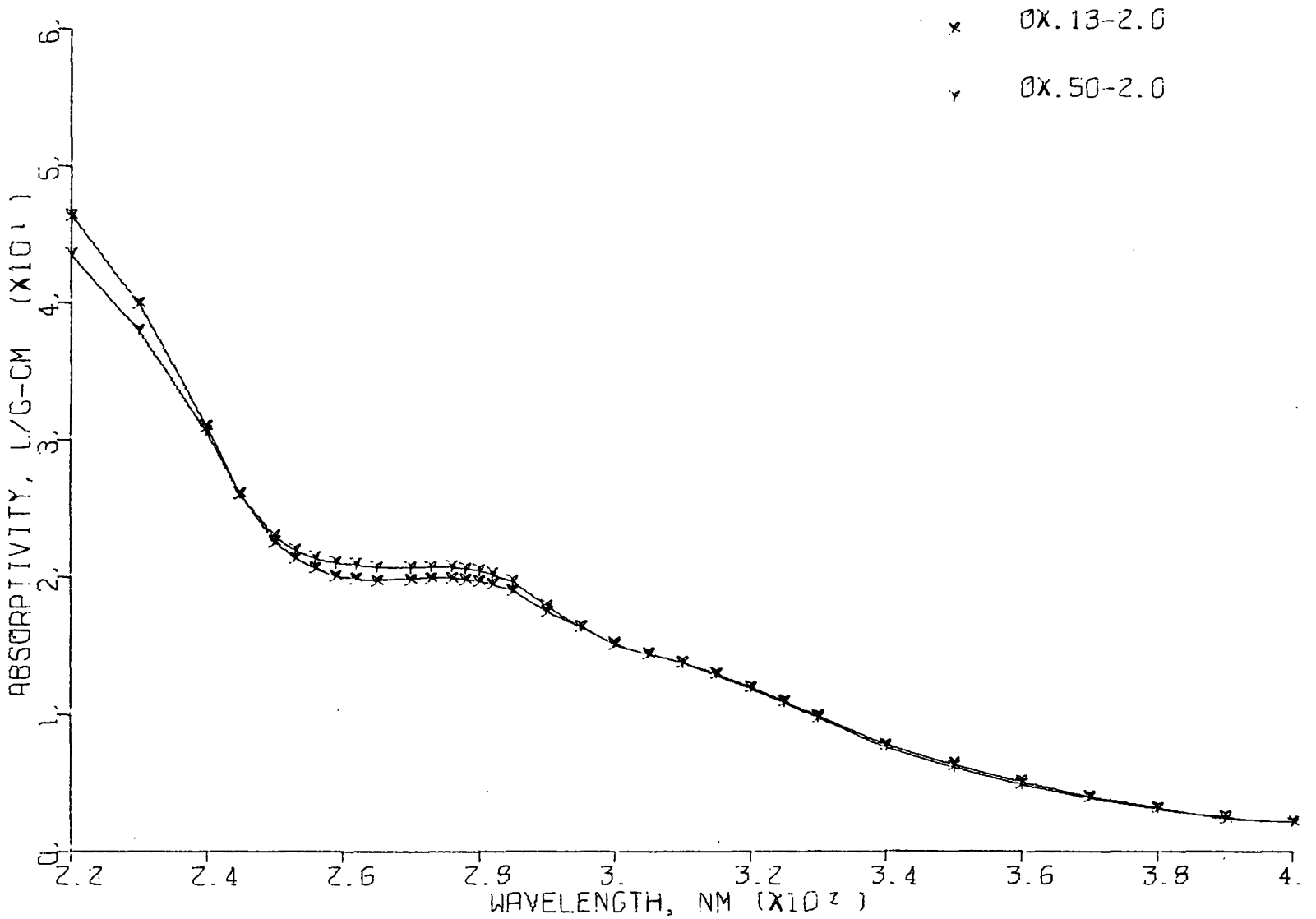


Figure 19. Comparison of Direct Ultraviolet Spectra of OX.13-2.0 and OX.50-2.0 Samples

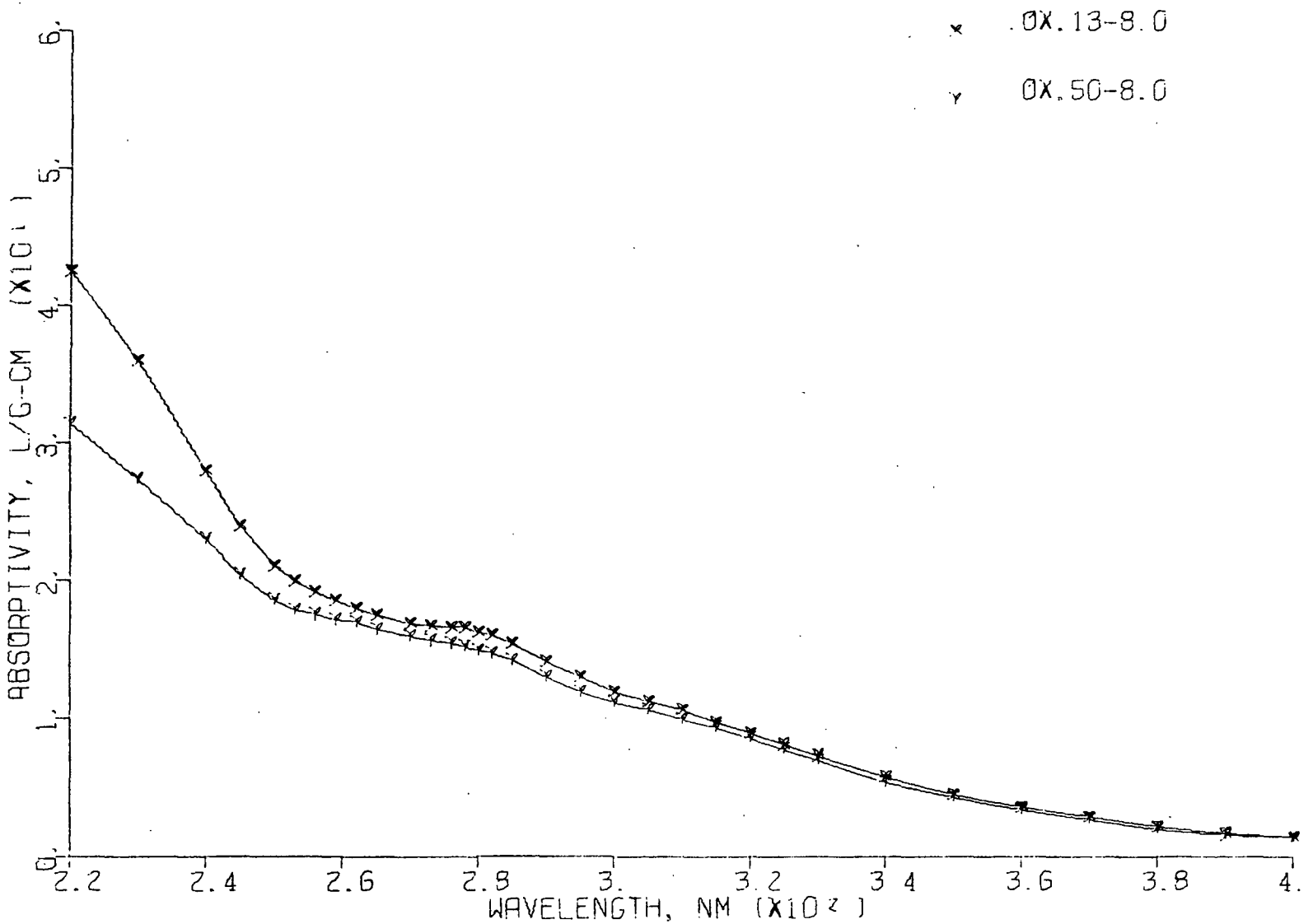


Figure 20. Comparison of Direct Ultraviolet Spectra of OX.13-8.0 and OX.50-8.0 Samples

experimental accuracy of the method there was no difference in UV absorption between samples oxidized for comparable lengths of time.

Ultraviolet Difference Spectra

The UV difference spectra of the oxidized lignins from the OX.13 and OX.50 series are compared in Fig. 21 and 22, respectively. The most significant feature of the UV difference spectra was the disappearance of the absorption bands at 250 and 300 nm with increasing time of reaction. These bands arise from the shift of primary and secondary bands of the benzene ring due to the presence of an ionized phenolic unit on the ring. Free phenolic hydroxyl contents are evaluated quantitatively from the intensity of the 250 nm band of the UV difference spectra in a subsequent section.

In addition to the disappearance of the 250 and 300 nm bands, there appeared to be a general trend toward an increase in the intensity of the broad absorption band at about 350 nm in the UV difference spectrum of the OX.50 series (Fig. 22). Absorption in this region of the difference spectrum has been assigned to α-carbonyl and α,β-ethylenic structures conjugated with an aromatic ring containing an ionized phenol (56). Neither of these types of structures would be expected to be formed under the oxidative conditions employed in this work.

INFRARED SPECTROSCOPY

Infrared spectra of the oxidized lignins and SM from the OX.13 and OX.50 series are compared in Fig. 23 and 24. Samples were prepared by freeze-drying at pH 9.0. Therefore, carboxyl groups in the lignin were present as the acid salt. Assignments of IR absorption bands for mildly isolated softwood lignins have been discussed previously (Table V).

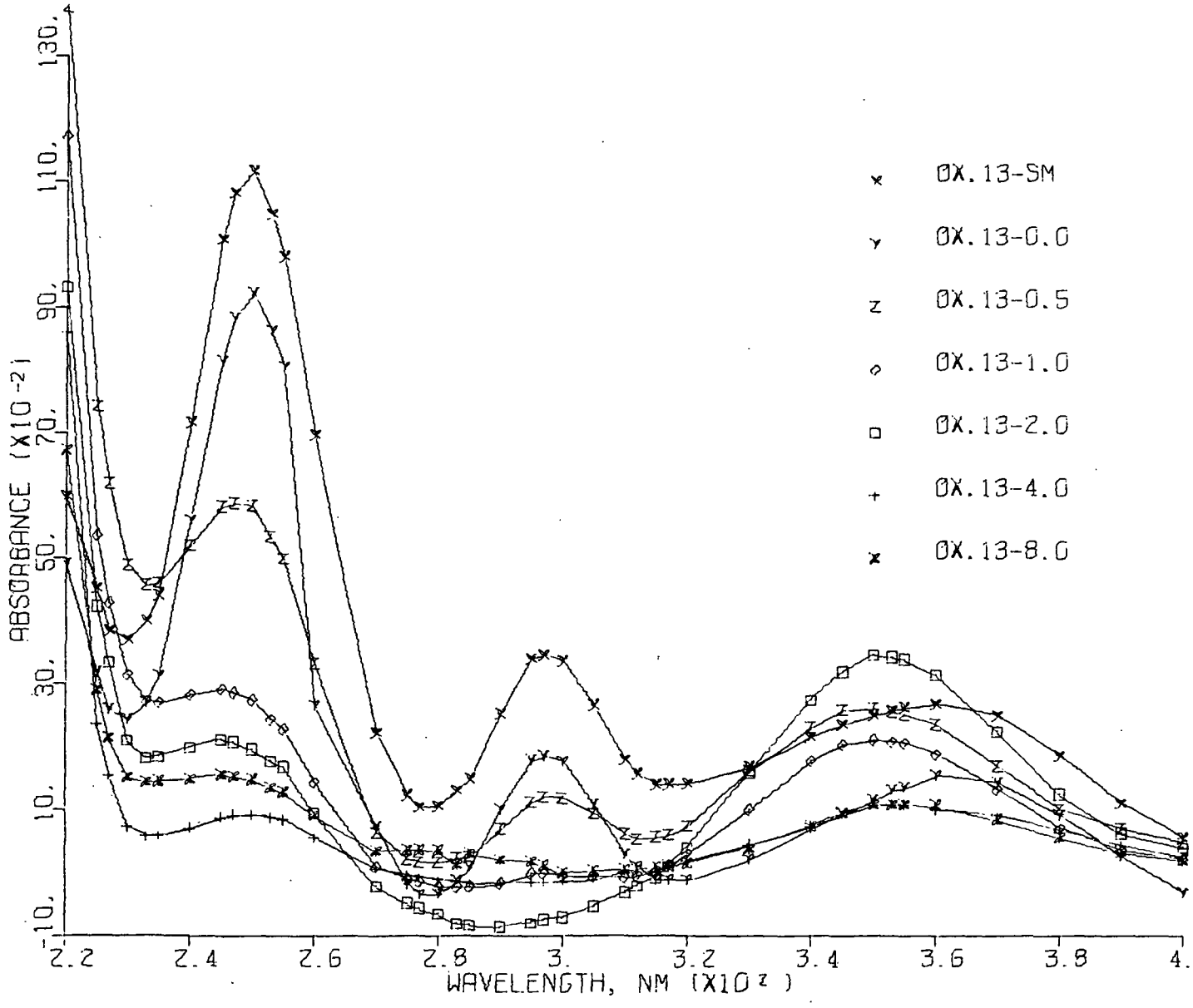


Figure 21. Comparison of Ultraviolet Difference Spectra of OX.13 Samples

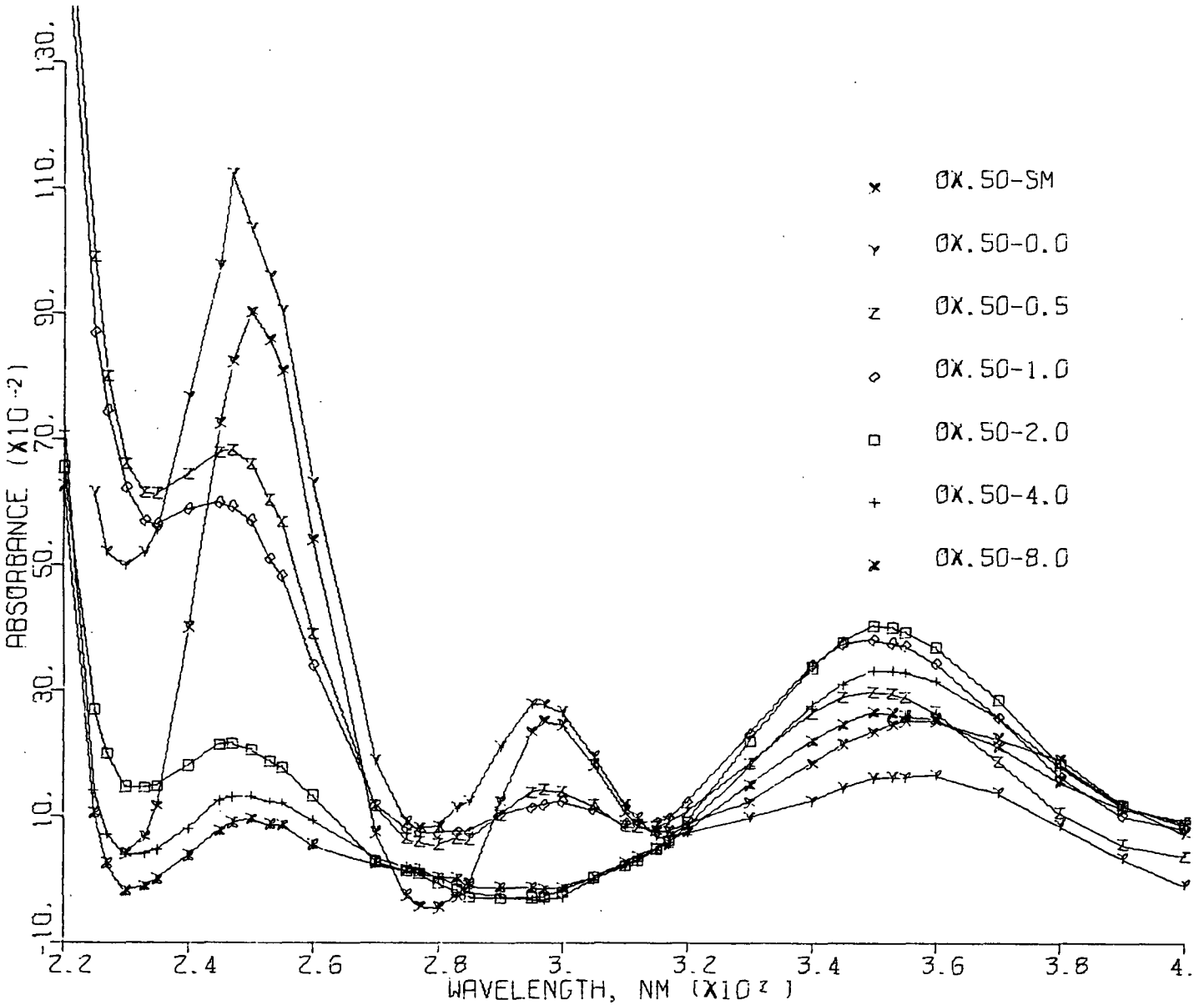


Figure 22. Comparison of Ultraviolet Difference Spectra of OX.50 Samples

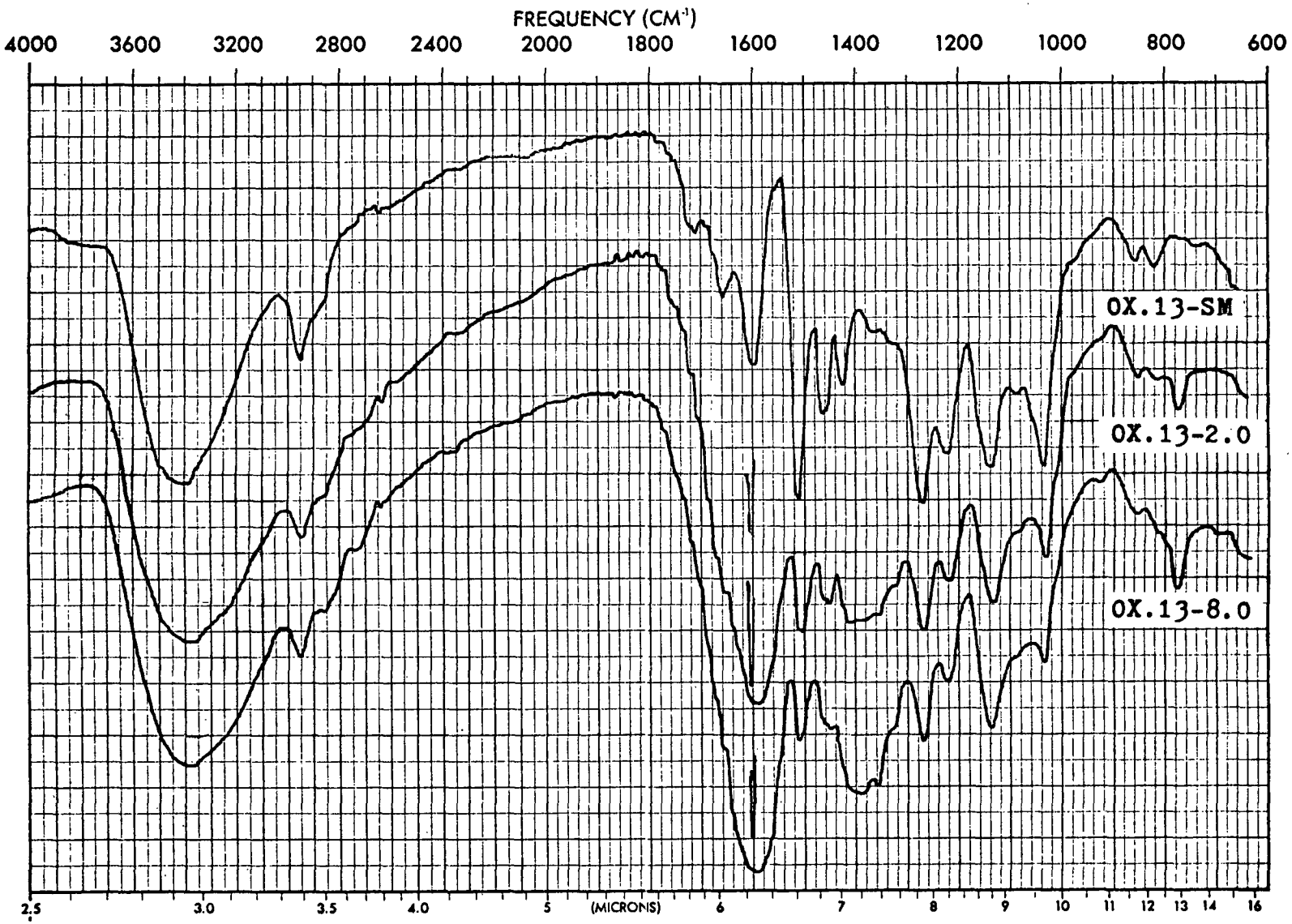


Figure 23. Comparison of Infrared Spectra of OX.13 Reaction Samples

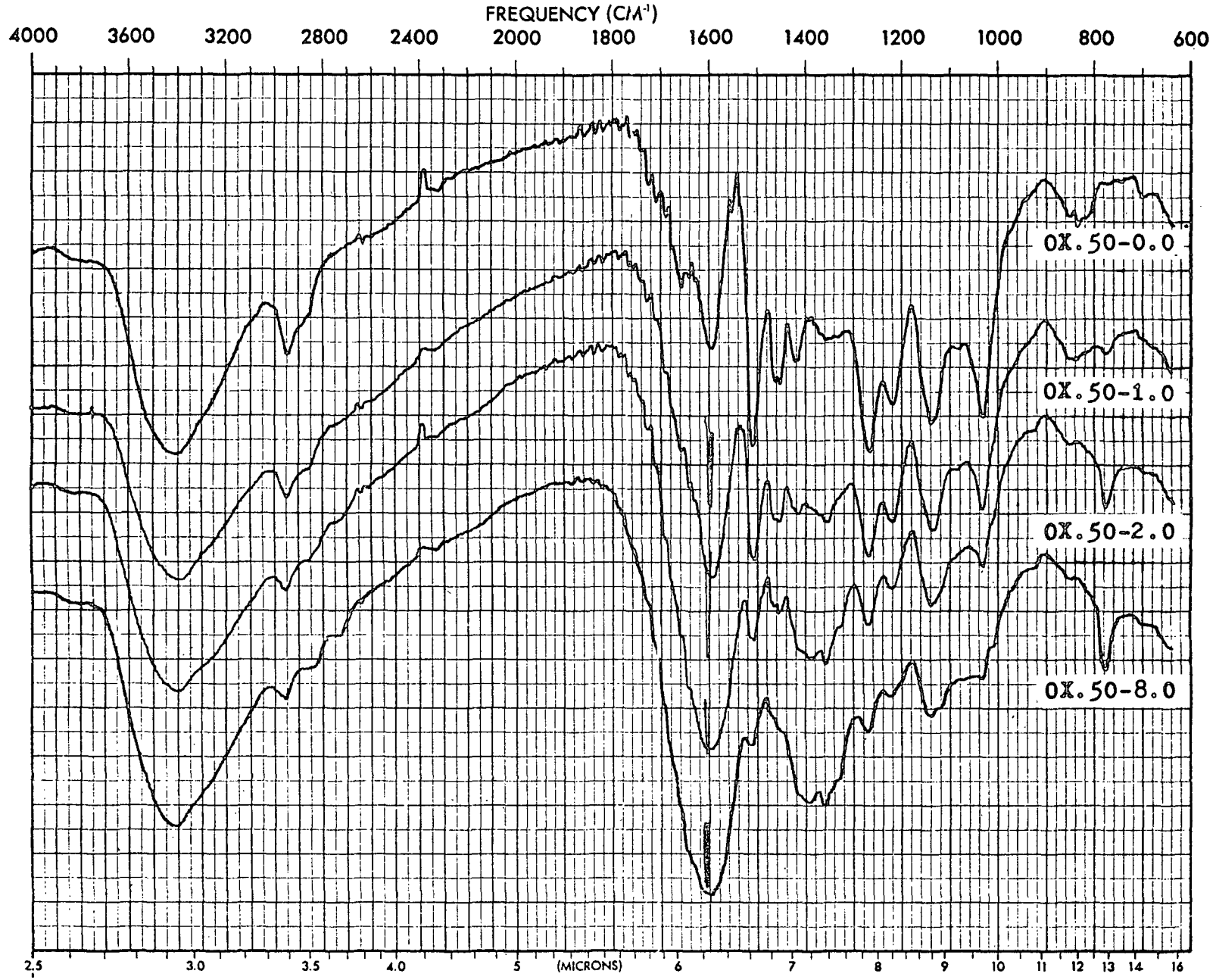


Figure 24. Comparison of Infrared Spectra of OX.50 Reaction Samples

Major changes in the IR spectra due to oxidation of the lignin with oxygen/alkali were:

1. An increase in intensity and broadening of the band at 3400 cm^{-1}, assigned to OH stretch.
2. An increase in intensity and broadening of a band at 1600 cm^{-1}, assigned to asymmetrical carboxylate anion stretch. The broadness and intensity of this band masked any change in the carbonyl absorption bands at 1660 and 1715 cm^{-1}.
3. The appearance of a broad band at about 1400 cm^{-1}, assigned to symmetrical stretch of the carboxylate anion.
4. An apparent decrease in the intensity of the band at 1030 cm^{-1}, assigned to aromatic C-H in-plane deformation and to C-O deformation of primary alcohols.
5. The appearance of a sharp band at 775 cm^{-1} with time of oxidation. A portion of the OX.50-8.0 sample was acidified to pH 3.0 and freeze-dried. IR spectra of the alkaline and acidified OX.50-8.0 samples are compared in Fig. 25. The disappearance of the band at 775 cm^{-1} in the acidified sample indicated that this band was related to aromatic out-of-plane deformations associated with the carboxylate anion, although no assignment could be made.
6. The disappearance of the pair of bands at 815 and 855 cm^{-1}, indicating loss of aromaticity with time of reaction.

Changes observed in the IR spectra of the oxidized lignins indicated that the major consequences of the oxidation of the lignin were formation of carboxylic acids and loss of aromaticity.

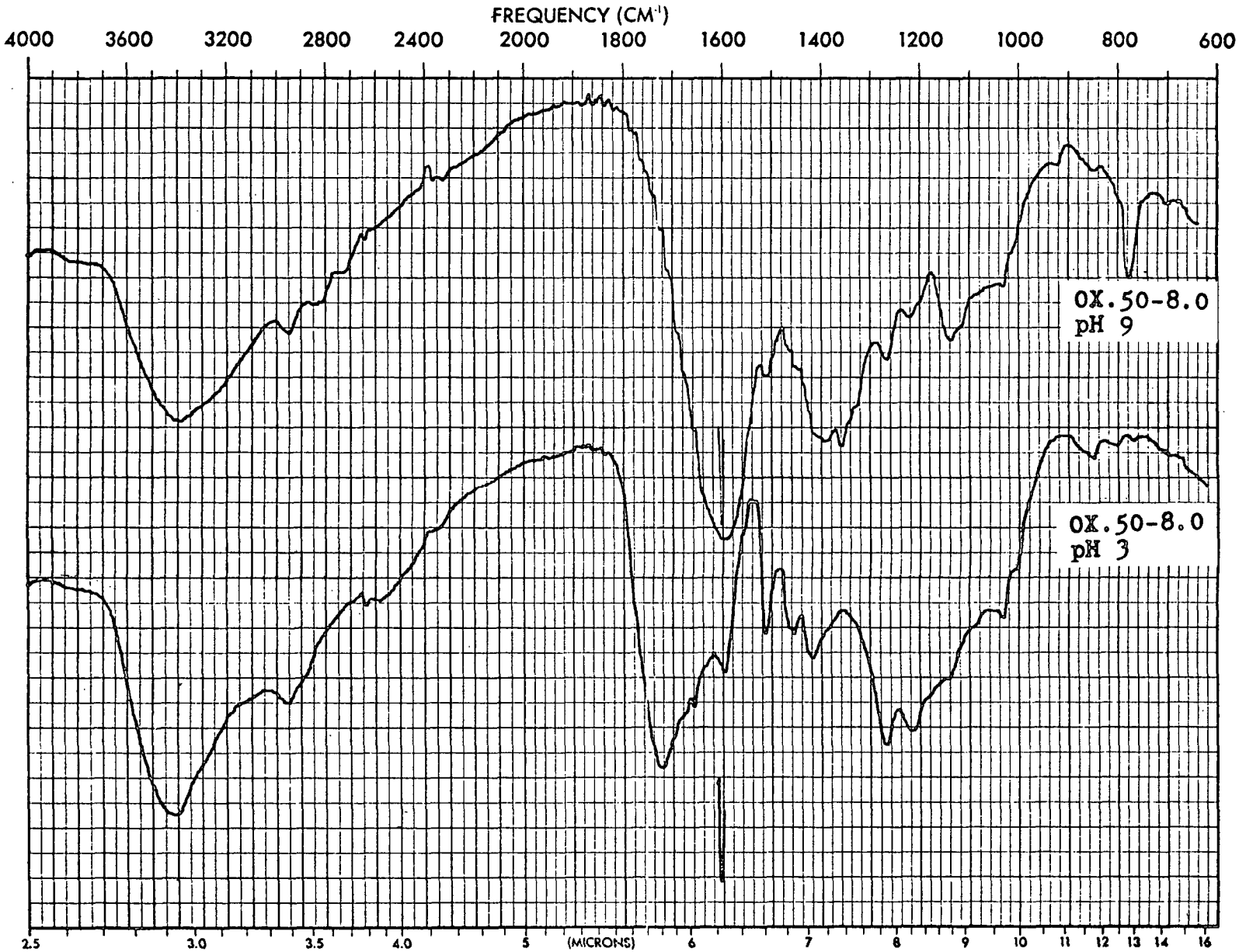


Figure 25. Comparison of Infrared Spectra of OX.50-8.0 Reaction Samples Isolated at a pH of 9 and at a pH of 3

PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

PMR spectra were run on samples of the acetylated lignins. There were some problems associated with preparation of the acetylated lignin which can best be discussed by briefly describing the acetylation procedure. Lignin samples (25 mg) were acetylated in solutions containing 1 ml of acetic anhydride and 1 ml of water-free pyridine. Whereas the DL, SM, and 0.0-hour samples were readily soluble in pyridine, the oxidized lignin samples were virtually insoluble. It was found that addition of 1/2 ml of dimethylformamide (DMF) greatly increased the solubility of the oxidized lignins, so DMF was added to all acetylation solutions. Following reaction for a minimum of 24 hours, the acetylation mixture was poured into distilled water and the acetylated lignin extracted with chloroform. The chloroform layer was washed with 1.0N HCl, then with water. The chloroform was removed under reduced pressure to give a yellow sirup which was taken up in deuterated chloroform for PMR analysis.

The chloroform solutions of the oxidized acetylated lignins were noticeably less colored than the solutions from the DL, SM, and 0.0-hour samples, indicating that less acetylated product had been obtained from the oxidized samples. That there was in fact less material in solution in the oxidized samples was confirmed by the PMR analysis, as it was necessary to accumulate 1000 pulses for the 8.0-hour sample to obtain a signal-to-noise ratio equivalent to that obtained with 200 pulses on the DL, SM, and 0.0-hour samples.

There was some residual DMF in all samples which was not removed by the washing procedure. However, the DMF signals were all very sharp and the integral could be readily corrected for these signals.

Integrals of the PMR spectra of the OX.13 and OX.50 series are presented in Tables XII and XIII, respectively. No effort was made to relate these integrals to the total protons per C_9 unit, as was done with the DL, because the C_9 unit structure may no longer be considered to be a valid representation of the oxidized lignins.

TABLE XII

INTEGRALS OF RANGES FOR CHEMICAL SHIFTS FOR PROTONS IN ACETYLATED OX.13 SERIES LIGNINS

Range[a]	Symbol	Total Integral, %						
		SM	0.0	0.5	1.0	2.0	4.0	8.0
1	H_a	17.1	18.2	16.7	17.5	15.9	17.3	18.6
2	H_α	5.6	5.5	6.0	7.9	8.9	6.1	8.2
3[b]	H_{OCH_3}	20.6	18.8	19.2	19.2	20.5	20.2	16.6
	$H_{\beta\gamma}$	16.5	19.7	17.7	17.1	15.4	12.8	18.2
4	$H_{\alpha'}$	5.5	4.7	7.0	6.0	5.4	5.7	6.1
5	H_{PhOAc}	7.1	6.4	6.3	6.2	6.7	5.3	3.9
6	H_{AlOAc}	21.8	22.3	22.2	21.2	21.8	25.2	24.4
7	$H_{\beta'\gamma'}$	5.8	4.5	5.1	4.9	5.2	7.3	4.1

[a] Shift ranges and assignments are tabulated in Table VII.

[b] H_{OCH_3} and $H_{\beta\gamma}$ were calculated from methoxyl contents and elemental analyses in Table X.

The integrals of the acetylated samples were marked by the lack of any consistent trend in the data. Based on model compound studies, one might predict that there would be a decrease in aromatic, methoxyl, and phenyl acetoxy protons as a function of time of reaction. There was, in fact, an apparent decrease in phenyl acetoxy protons in the OX.13 series, but the aromatic and methoxyl proton integrals appeared to remain relatively unchanged. In the OX.50 oxidation samples on the other hand, there was an apparent

decrease in aromatic and methoxyl protons, but the phenyl acetoxy proton integral appeared to remain relatively constant.

TABLE XIII

INTEGRALS OF RANGES FOR CHEMICAL SHIFTS FOR PROTONS IN ACETYLATED OX.50 SERIES LIGNINS

Range[a]	Symbol	Total Integral, %						
		SM	0.0	0.5	1.0	2.0	4.0	8.0
1	H_a	18.0	18.4	18.5	18.2	21.7	15.6	14.1
2	H_α	4.8	5.3	6.3	6.0	6.0	6.7	7.2
3[b]	H_{OCH_3}	21.0	18.0	20.8	19.8	17.7	16.8	12.4
	$H_{\beta\gamma}$	16.6	19.5	15.3	17.4	18.8	17.9	23.9
4	$H_{\alpha'}$	4.2	3.9	5.4	5.3	6.6	6.7	7.2
5	H_{PhOAc}	6.9	6.8	6.9	7.5	9.5	7.2	6.3
6	H_{AlOAc}	24.6	23.3	21.8	20.4	13.5	21.7	22.1
7	$H_{\beta'\gamma'}$	4.2	4.7	5.0	5.4	6.1	6.0	7.6

[a] Shift ranges and assignments are tabulated in Table VII.

[b] H_{OCH_3} and $H_{\beta\gamma}$ were calculated from methoxyl contents and elemental analyses in Table X.

It is believed that the lack of consistent trends in these data was related to the solubility problems associated with the preparation of the acetyl derivatives. The low solubility of the oxidized lignins in pyridine is thought to have been related to the higher molecular weights of these samples, relative to the SM and 0.0-hour samples.

The general shape of the PMR signals changed dramatically in going from the spectrum of the SM sample to the spectrum of the 8.0-hour sample, as illustrated in Fig. 26. Whereas the DL spectrum was composed of broad, ill-defined bands of overlapping signals, the signals in the OX.50-8.0 spectrum were in general much sharper in the acetoxyl and methoxyl regions. In addition,

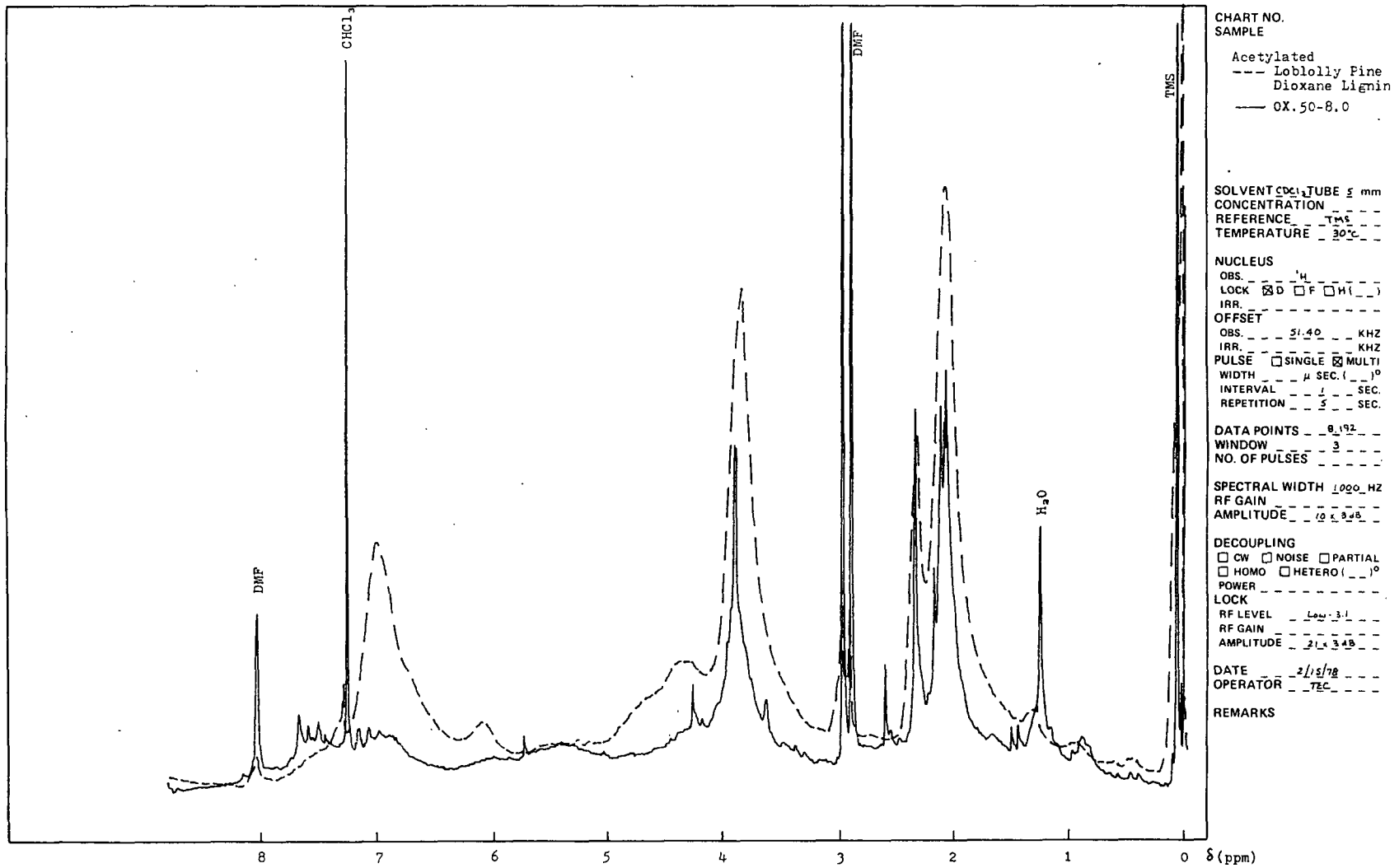


Figure 26. Comparison of PMR Spectra of Acetylated Loblolly Pine Dioxane Lignin and OX.50-8.0

a portion of the aromatic signal in the oxidized spectrum was shifted downfield, which would be consistent with either side-chain oxidation to α-carboxylic acids or ring-oxidation to muconic acids.

PHENOLIC HYDROXYL

Changes in phenolic hydroxyl content as a function of time of oxidation were of considerable interest in this investigation since the free phenolic hydroxyl group has been implicated in model compound studies as an initiating site in degradation of lignin with oxygen in alkaline medium. Phenolic hydroxyl contents of the OX.13 and OX.50 samples were calculated from UV difference spectra (Table XIV) according to the method of Wexler (83) and from integrals of the acetylated PMR spectra (Table XV) according to the procedure of Morohoshi and Sakakibara (79).

TABLE XIV

FREE PHENOLIC HYDROXYL CONTENTS OF OXIDIZED LIGNINS CALCULATED FROM UV DIFFERENCE SPECTRA[a]

Sample Number	Oxidation Run OX.13 Phenolic Hydroxyl, %	Oxidation Run OX.50 Phenolic Hydroxyl, %
SM	2.42	2.43
0.0	1.97	1.93
0.5	0.79	0.74
1.0	0.19	0.55
2.0	0.24	0.33
4.0	0.00	0.29
8.0	0.11	0.28

[a]Calculated from UV difference spectra in Fig. 21 and 22.

TABLE XV

FREE PHENOLIC HYDROXYL CONTENTS OF OXIDIZED LIGNINS CALCULATED FROM THE INTEGRALS OF PMR SPECTRA[a]

Sample Number	Oxidation Run OX.13 Phenolic Hydroxyl, %	Oxidation Run OX.50 Phenolic Hydroxyl, %
SM	3.0	2.8
0.0	2.8	3.0
0.5	--	2.6
1.0	2.3	2.8
2.0	2.3	3.4
4.0	1.6	1.9
8.0	1.2	1.7

[a]Calculated from PMR integrals in Tables XII and XIII and from elemental analyses in Table X.

Based on model compound studies of the degradation of lignin with O/A, phenolic hydroxyl groups may be formed, for example, by cleavage of aryl-ether linkages to guaiacol-type structures, or consumed, for example, through aromatic ring opening reactions to yield muconic acid structures. Phenolic hydroxyl contents calculated from UV difference spectra indicated that, at both concentrations of alkali examined, the phenolic hydroxyl content decreased rapidly in the early stages of oxidation. The absolute values computed from UV difference spectra may be somewhat open to question here, as the empirical factor calculated by Wexler (83) for computing phenolic hydroxyl was based on nonoxidized lignin model compounds. At the lower phenolic hydroxyl levels (below 0.50%) the accuracy of the UV difference method is probably no better than ± 0.20%. In spite of this qualification, however, the disappearance of the bands at 250 nm and at 300 nm in the UV difference spectra (Fig. 21 and 22) of the highly oxidized samples lends strong qualitative evidence for destruction of phenolic hydroxyls in the oxidative degradation of the lignin.

With the exception of the OX.13-4.0 sample none of the phenolic hydroxyl contents calculated from the UV difference spectra were zero, suggesting the initial presence of or the formation of relatively stable phenolic hydroxyl containing structures. In addition, after 1 hour of oxidation the phenolic hydroxyl content of the OX.50 samples appeared to be somewhat higher than the corresponding OX.13 samples, although the differences were barely significant.

Phenolic hydroxyl contents calculated from PMR spectra of the acetylated samples likewise decreased as a function of time of oxidation. However, the absolute values calculated remained considerably higher than phenolic hydroxyl levels computed from the UV difference spectra.

The PMR method for determination of phenolic hydroxyl measures the proton distribution of the acetylated lignin in deuterated chloroform. As has been mentioned, there was less acetylated product dissolved in the chloroform for the oxidized lignin samples than for the SM and 0.0-hour samples. The higher phenolic hydroxyl values computed from the PMR integrals, relative to the values calculated from UV difference spectra, suggest that in the oxidized samples, the acetylated fraction containing relatively higher levels of phenyl acetoxy groups was preferentially dissolved in the chloroform.

As has been mentioned in the Introduction, the most widely accepted mechanism for the initiation of lignin degradation by oxygen in alkaline medium proceeds through an electron transfer from the phenolate anion to oxygen to form a phenoxy radical and a hydroperoxy radical (Fig. 1). Destruction of phenolic hydroxyl groups under O/A pulping conditions in this work would, therefore, indicate that secondary degradation mechanisms predominate after the first hour of oxidation.

CARBONYL

Carbonyl contents of the OX.13 and OX.50 samples determined by oximation (86) are presented in Table XVI.

TABLE XVI

CARBONYL CONTENTS OF OXIDIZED LIGNINS

Sample Number	Oxidation Run OX.13 Carbonyl, %	Oxidation Run OX.50 Carbonyl, %
SM	2.63	--
0.0	2.06	1.71
0.5	0.40	0.54
1.0	0.17	--
2.0	0.74	0.00
4.0	0.40	0.00
8.0	0.00	--

As has been noted for other functional groups, there was a significant decrease in carbonyl as a result of bringing the reactor to temperature. Carbonyl contents at both concentrations of alkali decreased rapidly in the early stages of oxidation. The contribution of α-carbonyls to the reactivity of the side-chain was discussed in the Introduction. Loss of carbonyls in the first hour of reaction would suggest that, as with the phenolic hydroxyl-related mechanism, secondary degradation mechanisms predominate in the later stages of oxidation.

CARBOXYL

The carboxyl content and molecular weight of an oxidized lignin have been suggested as the major factors which contribute to the solubilization of the degraded lignin. Sakai and Kishimoto (94) estimated that 0.25 eq $COOH/C_9$ unit (6.2%, w/w) was the minimum carboxyl content necessary for

dissolution of a PAA-oxidized pine DL in water. Of the samples isolated in the present investigation only the OX.50-8.0 sample (18.9% COOH, w/w) was completely soluble in water.

Carboxyl contents of the OX.13 and OX.50 samples were estimated by potentiometric titration. Results are summarized in Table XVII. Both oxidation runs were characterized by a rapid initial increase in carboxyl with a slower increase at longer oxidation times. For a given time of oxidation the OX.50 series had a consistently higher carboxyl content, indicating more extensive degradation of the lignin.

TABLE XVII

CARBOXYL CONTENTS OF OXIDIZED LIGNINS

Sample Number	Oxidation Run OX.13 Carboxyl, %	Oxidation Run OX.50 Carboxyl, %
SM	0.81	0.0
0.0	0.46	0.60
0.5	4.6	8.0
1.0	7.7	9.2
2.0	9.5	14.1
4.0	10.9	17.8
8.0	14.5	18.9

MOLECULAR WEIGHT DISTRIBUTION

Several studies on the molecular weight distributions of oxidized lignins have been reported (47,95-97). Kopnin and Chupka (95) oxidized aspenwood DL for two hours under an oxygen pressure of 10 atmospheres (@ 20°C) in 0.1N Na_2CO_3 at a temperature of 140°C. The molecular weight distribution of the oxidized lignin was determined on Sephadex G-75 using dimethylsulfoxide as eluant. The molecular weight distribution of the oxidized lignin showed a

shift to lower molecular weight relative to the DL starting material. No high molecular weight peak was observed.

Katuscakova and Oltus (96) prepared an isolated lignin by extraction of spruce wood meal with 2-chloro-ethanol. The isolated lignin was oxidized with oxygen at a pressure of 8 atmospheres in 2N NaOH at a temperature of 95-100°C. The molecular weight distribution was determined on Sephadex G-25 with 2N NaOH as eluant. A high and a low molecular weight peak were observed after an oxidation time of 20 minutes. The size of the low molecular weight fraction increased with time of oxidation with a corresponding decrease in the size of the high molecular weight fraction.

Sogo and Hata (97) oxidized Japanese red pine DL at an oxygen pressure of 100 psi (@ 20°C) in alkali containing NaOH and $NaHCO_3$ at various pH's and temperatures. The molecular weight distributions were determined on Sephadex G-75 with alkali at a pH of 10.5 used as eluant. At a pH of 13.5 they observed formation of low molecular weight degradation products, even at temperatures as low as 70°C. Higher temperatures (120°C, 140°C) yielded correspondingly greater amounts of low molecular weight products. At a pH of 8.5 they observed that some high molecular weight material remained, even after cooking times of 2 hours at temperatures of 140°C.

The most extensive study of the molecular weights and molecular weight distributions of oxidized lignins has been the work of Karna (47). Although the lignin systems studied by Karna were very different from the one used in this investigation, the results obtained by Karna merit discussion in some detail. He studied the molecular weight properties of two lignin preparations, designated as soda lignin and as oxygen lignin. Soda lignin was that lignin which became soluble after the soda pulping of Douglas-fir chips. It was assumed by Karna that the soda lignin was representative of

the lignin remaining in the wood pulp following the soda process. The oxygen lignin was isolated by acid precipitation of spent liquors from the 1-stage oxygen pulping (pH 7-9) of Douglas-fir chips. Molecular weight distributions were determined on gel columns containing Sephadex grades G-15, G-25 and G-100 arranged in series, using 0.1N NaOH as eluant. Elution was monitored by monochromatic light absorption at 280 nm. A column calibration curve was prepared using three fractionated kraft lignin samples of known molecular weight.

Molecular weight distributions of the OX.13 and the OX.50 samples studied in this investigation were determined on gel columns containing Sephadex G-50 or Sephadex G-75, using formamide as eluant. Formamide was reported by Brown, et al. (91) to have negligible differential solvation and gel interaction effects, whereas in aqueous solvents, these effects had a pronounced role in separation. Sample addition to the columns was 50 mg of lignin dissolved in 3 ml of formamide in each case. Elution was monitored by monochromatic light absorption at 280 nm. Interpretation of molecular weight distribution curves based on UV absorption must be qualified, as light absorption by the eluant is dependent on the absorption coefficient of the solute as well as the concentration of material in solution. No calibration of the high molecular weight region of the elution spectrum was made, as monodisperse fractions of high molecular weight were not commercially available.

The molecular weight distributions of the OX.13 samples are compared in Fig. 27, determined on Sephadex G-50. Two lignin model compounds, vanillic acid (MW = 168) and 1-(3,4-dimethoxyphenyl)-2-(2-methoxy-4-methylphenoxy)-1-propanone (MW = 330) were eluted from the column to calibrate the low molecular weight region of the curve. The elution volume at which maximum absorbance of the model compounds was observed is indicated.

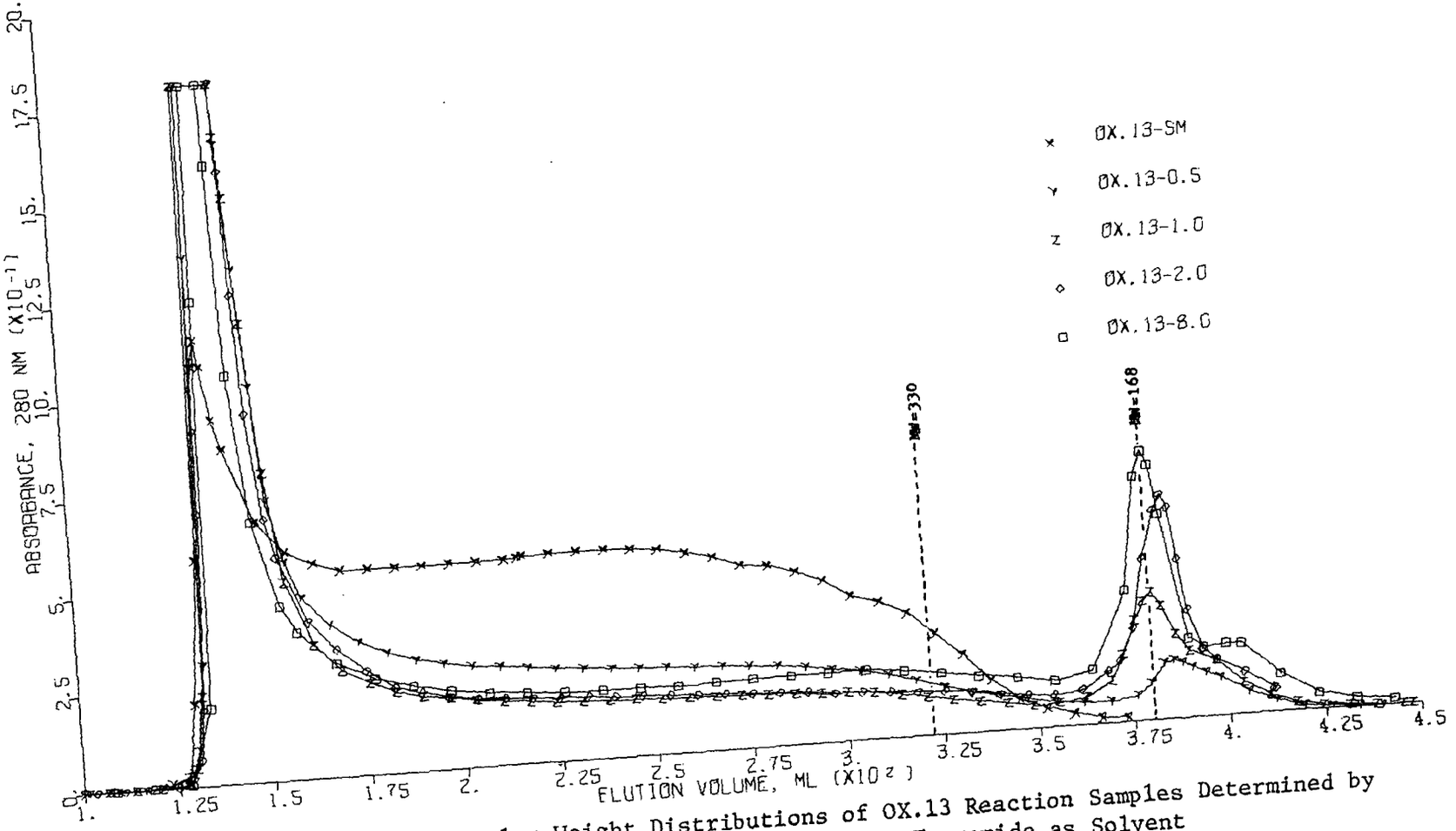


Figure 27. Comparison of Molecular Weight Distributions of OX.13 Reaction Samples Determined by Chromatography on a Column of Sephadex G-50, with Formamide as Solvent

Elution curves were bimodal, exhibiting both a low molecular weight fraction and a high molecular weight fraction containing material too large to be resolved by the column. There was a general decrease in the absorbance of the oxidized samples in the intermediate molecular weight region relative to the SM sample. These results may be interpreted to indicate that there was rapid cross-linking of the lignin in the initial stages of reaction. The low molecular weight peak at an elution volume of 380 ml indicated formation of degradation products with a molecular weight close to that of vanillic acid (MW = 168). The formation of a high molecular weight fraction through cross-linking reactions, followed by degradation to low molecular weight products, is consistent with the results of Katuscakova and Oltus (96).

Karna (47) found that mild oxidation of soda lignin (60°C, 1 atm O_2, 12 hr) at a pH of 10 resulted in an increase in the fraction with a molecular weight over 50,000 with a corresponding decrease in the low molecular weight fraction. Oxidation at pH 14, on the other hand, caused a general decrease in molecular weight and an increase in the size of the low molecular weight fraction. When reaction temperatures and pressures were increased to more accurately represent pulping conditions, Karna observed that oxidation of the soda lignin at pH 9 indicated no increase in molecular weight. At a pH of 14 he observed a well defined maximum at an elution volume corresponding to a molecular weight of 160. This fraction was attributed to vanillin, vanillic acid, and acetoguaiacone. Unlike the present study, however, Karna found that the low molecular weight fraction was not stable toward further oxidation and disappeared after oxidation for 4 hours.

The exclusion limit for Sephadex G-50 is listed at approximately 10,000 for dextrans and at approximately 30,000 for globular proteins in aqueous systems. In order to attempt to relate the relative intensity of the high

molecular weight peaks for the different oxidation samples, a column was prepared with Sephadex G-75, which has a listed exclusion limit of approximately 50,000 for dextrans and 70,000 for globular proteins in aqueous systems.

The molecular weight distributions of the OX.50 samples are compared in Fig. 28, determined on Sephadex G-75. As with the OX.13 samples, the elution curves were bimodal. The increase in the size of the excluded portion of the OX.50-0.0 sample relative to the OX.50-SM sample suggested that some cross-linking of the lignin occurred in the process of bringing the reactor to temperature. Comparison of the 0.0- and 1.0-hour samples demonstrated the formation of a large high molecular weight fraction and some low molecular weight material at the expense of the intermediate molecular weight portion on charging the reactor with oxygen and alkali. Comparison of the 1.0-, 2.0-, and 8.0-hour samples clearly indicated a decrease in the size of the high molecular weight portion as a function of time of reaction.

Molecular weight distributions of the 2.0- and 8.0-hour samples from the OX.13 and OX.50 runs are compared in Fig. 29 and 30, respectively, using Sephadex G-75. The results indicated that for a given time of oxidation, lignin oxidized at the higher alkali concentration was more extensively degraded.

A sample of DL and one of the OX.50-2.0 lignin were also separated on a column of Sephadex G-100 (exclusion limit for dextrans = 100,000, for globular proteins = 150,000 in aqueous solvents) in order to try and obtain some measure of the upper limit of the oxidized material. The elution curves for the two samples are compared in Fig. 31. A small amount of the DL was still excluded from the column. There was again a large high molecular weight peak in the oxidized sample, indicating the presence of material too large to resolve with the column. The high molecular weight fraction was

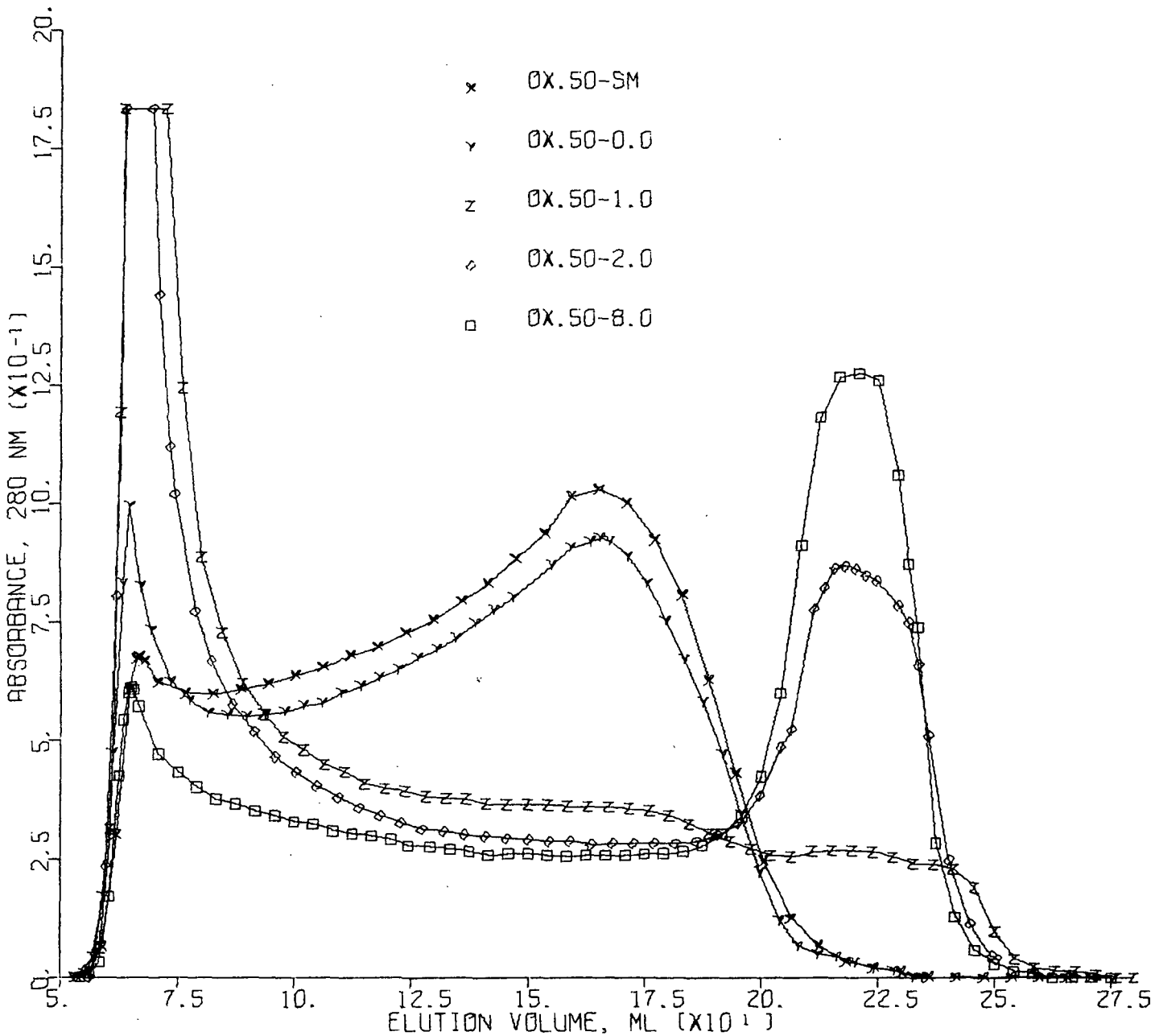


Figure 28. Comparison of Molecular Weight Distributions of OX.50 Reaction Samples Determined by Chromatography on a Column of Sephadex G-75, with Formamide as Solvent

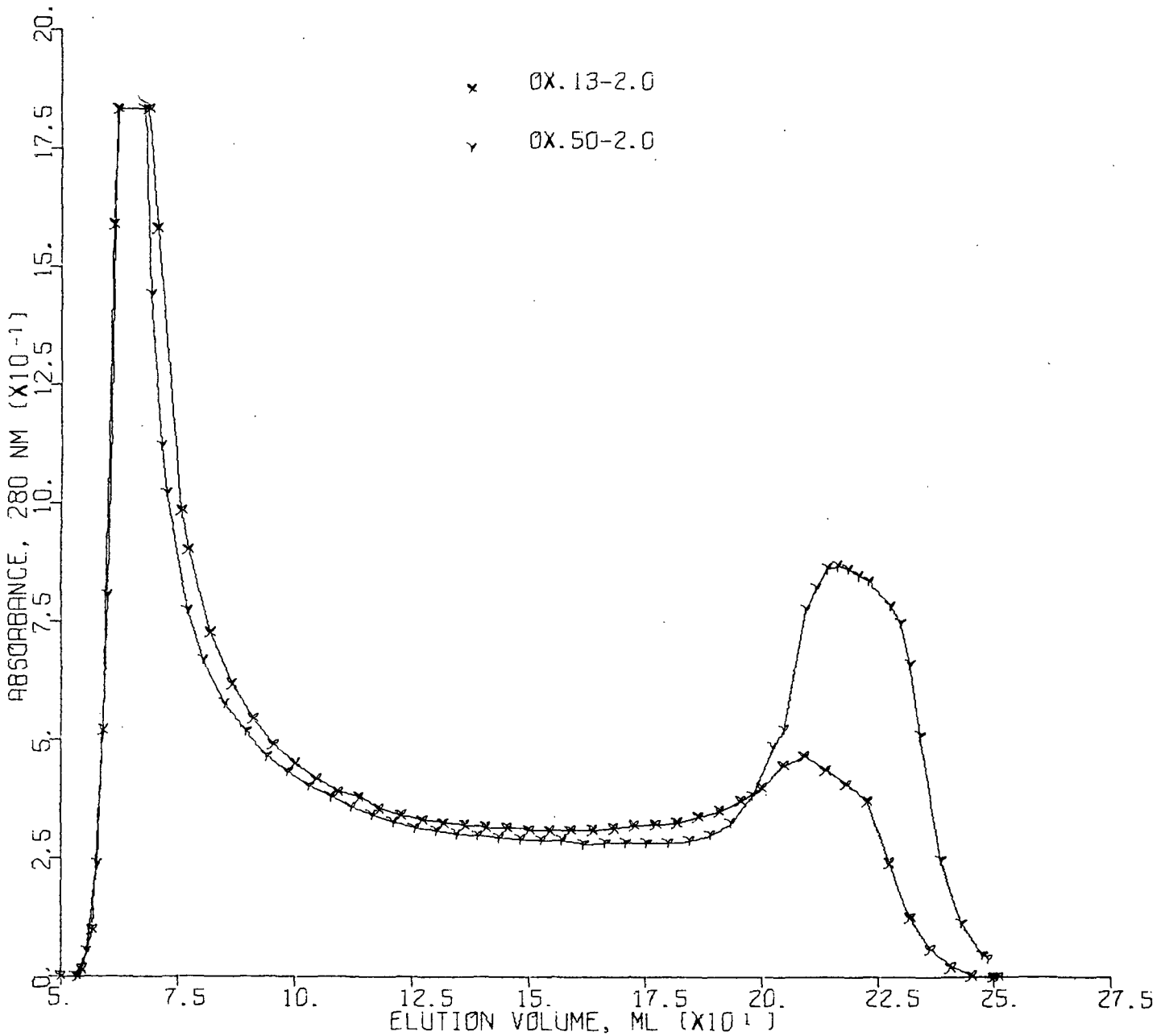


Figure 29. Comparison of Molecular Weight Distributions of OX.13-2.0 and OX.50-2.0 Reaction Samples Determined by Chromatography on a Column of Sephadex G-75, with Formamide as Solvent

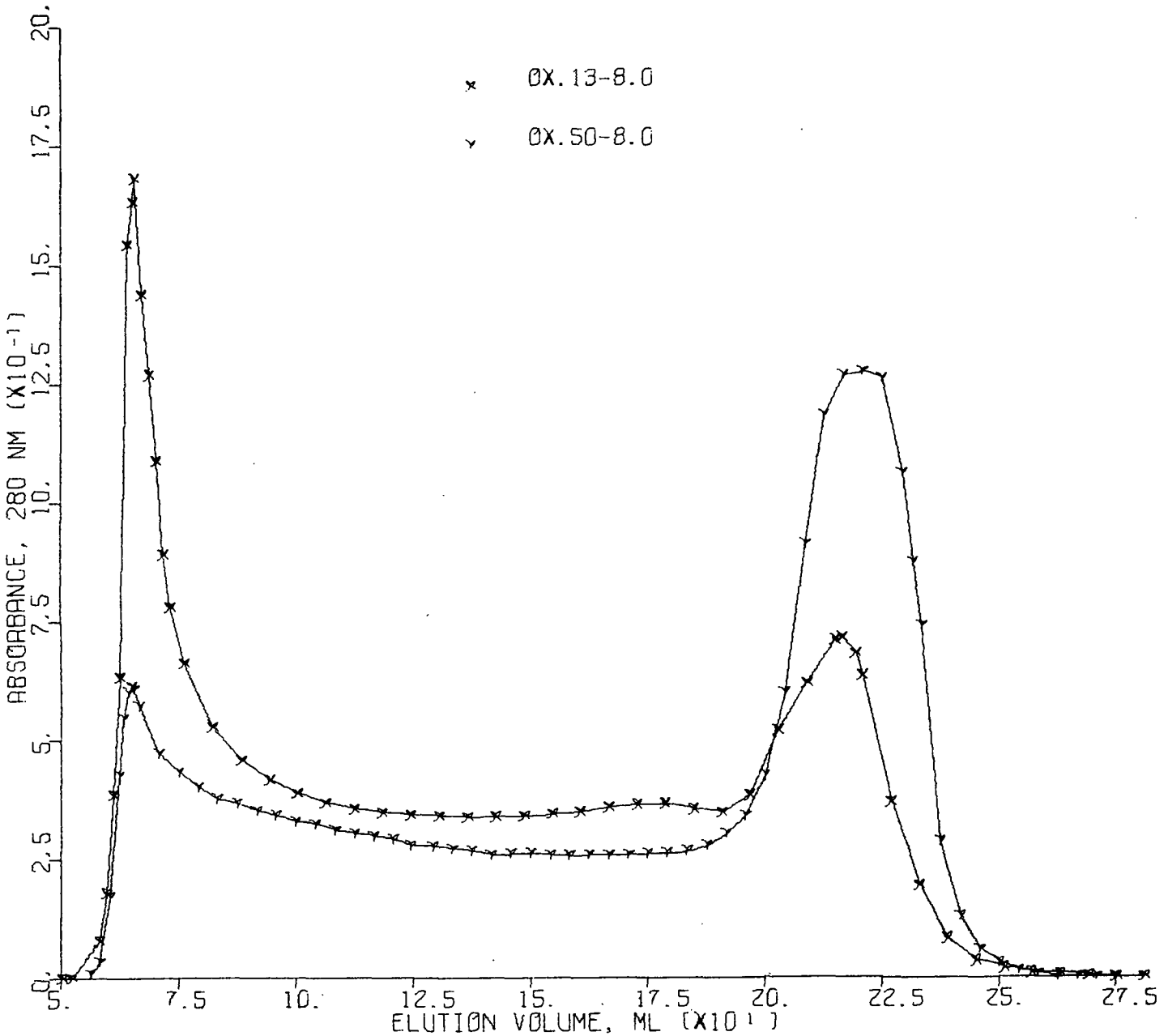


Figure 30. Comparison of Molecular Weight Distributions of OX.13-8.0 and OX.50-8.0 Reaction Samples Determined by Chromatography on a Column of Sephadex G-75, with Formamide as Solvent

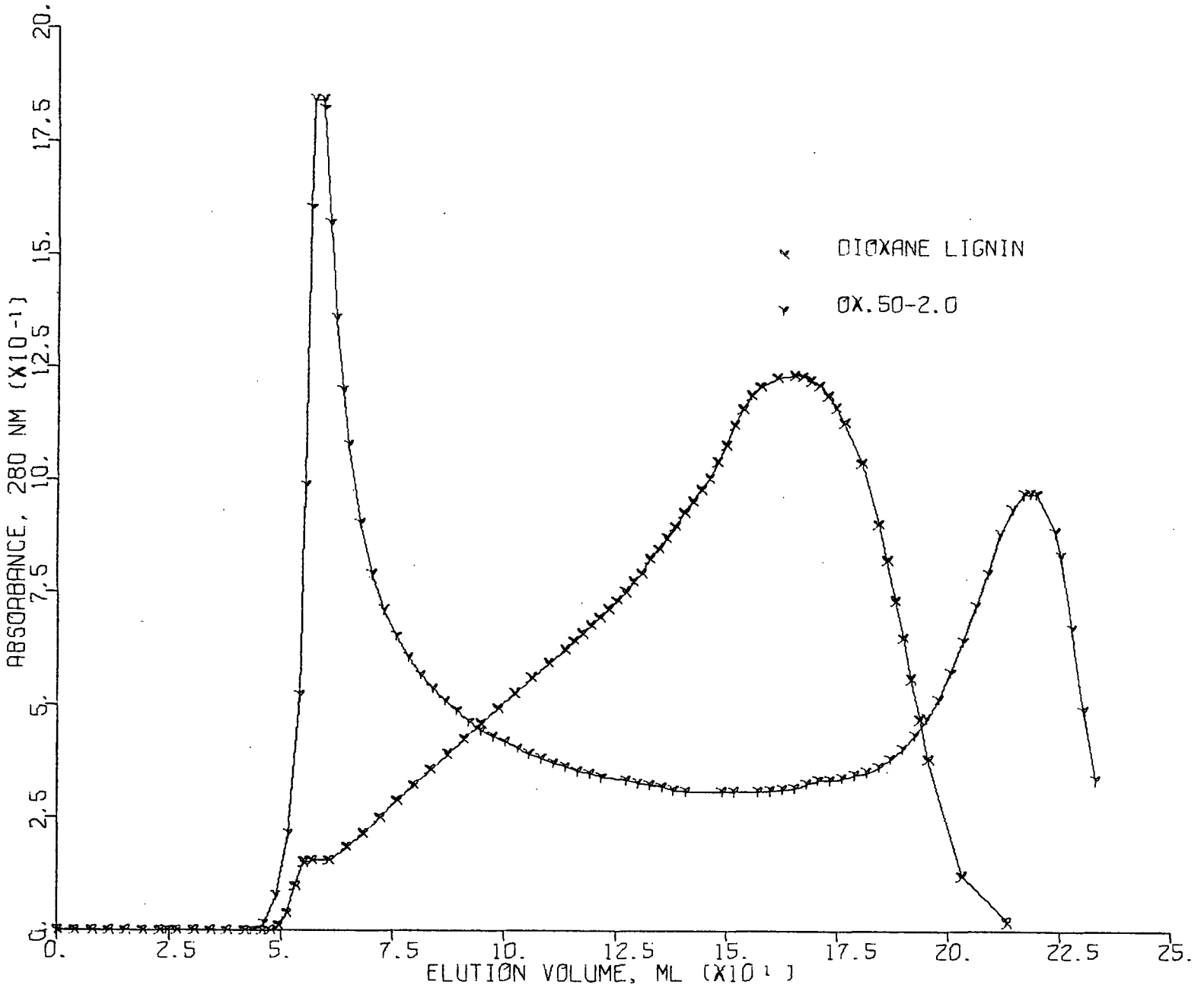


Figure 31. Comparison of Molecular Weight Distributions of Dioxane Lignin and OX.50-2.0 Samples Determined by Chromatography on a Column of Sephadex G-100

isolated, freeze-dried (freeze-dried weight = 7 mg out of 50 mg originally placed on the column), and analyzed for molecular weight by the meniscus depletion method on an ultracentrifuge. The high molecular weight fraction taken for analysis is indicated in Fig. 31. The weight average molecular weight was estimated to be 78,000 and the number average molecular weight was estimated to be 64,000 by the meniscus depletion method. Analytical methods for determination of molecular weight are discussed in Appendix IV.

SUMMARY — OXIDATION OF DIOXANE LIGNIN WITH OXYGEN/ALKALI — ROLE OF ALKALI CONCENTRATION ON CHANGES IN THE CHEMICAL PROPERTIES OF THE LIGNIN

Oxidation of DL at two concentrations of sodium carbonate (0.13 and 0.50N) indicated that the lignin samples oxidized at the higher alkali concentration were more extensively degraded for a given time of oxidation. Results may be summarized as follows:

1. Methoxyl contents of both oxidation series decreased throughout the oxidation, with a somewhat higher rate of demethylation in the early stages of reaction. At a given time of oxidation, the OX.50 sample was more extensively demethylated than the corresponding OX.13 sample.
2. Direct UV spectra indicated that there was an increase in absorptivity at 260 and 300 nm. These changes, together with the lack of decreased absorptivity at 280 nm which would have been expected due to loss of methoxyl and phenolic hydroxyl, suggested that the formation of biphenyl linkages may have been an important reaction pathway in the early stages of oxidation.
3. Infrared spectra indicated that a major reaction in both oxidation series was an increase in carboxylic acid groups. The disappearance

of C-H bending bands between 800 and 900 cm^{-1} indicated loss of aromaticity with time of oxidation.

4. In both oxidation series, phenolic hydroxyl was rapidly consumed in the initial stages of reaction.
5. In both oxidation systems, carbonyl was rapidly consumed in the initial stages of reaction.
6. In both oxidation series, there was a rapid initial increase in carboxyl content. The OX.50 series had a higher carboxyl content for a given time of oxidation than did the OX.13 series, indicating more extensive degradation of the OX.50 series samples.
7. Interpretation of PMR spectra of the oxidized lignins was obscured by incomplete dissolution of the acetylated samples. Integrals of the PMR spectra of the OX.50 samples appeared to support the loss of aromaticity with time of oxidation.
8. Changes in molecular weight distributions for both oxidation series showed a similar pattern — initial formation of a high molecular weight cross-linked lignin which subsequently fragmented to low molecular weight products with further oxidation. For a given time of oxidation, the OX.50 sample appeared to contain more low molecular weight degradation product than the corresponding OX.13 sample.

The greater degradation of the lignin in 0.50N Na_2CO_3 was somewhat surprising, as initial pH values for the two oxidation series differed by only 0.15 pH unit. The more extensive degradation at higher alkali suggests that the OX.13 degradation series was alkali limited.

The disappearance of phenolic hydroxyl with corresponding formation of a high molecular weight fraction is consistent with the involvement of phenolic

hydroxyl groups in the formation of the cross-linked material. Dimerization of phenoxy radicals is one way in which this may occur. The formation of biphenyl linkages in the oxidized lignin is supported by the increased UV absorbance at 260 and 300 nm and the lack of any decrease in absorbance at 280 nm with time of oxidation.

OXIDATION OF DIOXANE LIGNIN WITH OXYGEN/ALKALI — COMPARATIVE STUDIES ON A PEROXYACETIC ACID-MODIFIED LIGNIN

As was discussed in the preceding section, oxidation of the DL with O/A leads to rapid loss of phenolic hydroxyl and carbonyl groups. Based on proposed mechanisms for degradation of lignin model compounds with O/A, one would expect that an increase in phenolic hydroxyl and/or α-carbonyl content in lignin would result in an increase in the rate of lignin fragmentation. Peroxyacetic acid (PAA) has been reported to introduce these types of functional groups to lignin. The relevant chemistry of PAA has been reviewed in the Introduction.

One-gram samples of DL were oxidized with PAA in preliminary experiments in order to attempt to increase phenolic hydroxyl and/or α-carbonyl contents of the lignin. In order to increase these functional groups with a minimum amount of other degradation of the lignin, a low molar ratio of oxidant to lignin was used.

Lignin samples were allowed to react until all of the PAA had been consumed to avoid the need for addition of reducing agents. Following oxidation with PAA, the pretreated lignin was freeze-dried and phenolic hydroxyl content determined from the UV difference spectra according to the procedure of Wexler (83). Concentrations of PAA and hydrogen peroxide (from PAA degradation) were determined by the method of Sully and Williams

(99). Results of the preliminary experiments on PAA pretreatment of the DL are summarized in Table XVIII.

TABLE XVIII

PEROXYACETIC ACID PRETREATMENT OF DIOXANE LIGNIN

PAA/Lignin[a], mole ratio	H_2O_2/Lignin[a], mole ratio	Phenolic Hydroxyl, %
0.0	0.0	2.44
0.101	0.0098	2.44
0.306	0.030	2.77
0.506	0.049	2.85
1.01	0.098	2.70

[a]Formula weight of DL = 188.9.

The small increase in phenolic content probably arose from the fact that the hydroxylated aromatic compound is more reactive than the starting material and is oxidized further to other products. The maximum phenolic hydroxyl content was observed in the sample oxidized at a molar ratio of 0.506 PAA/lignin. Based on these results, a molar ratio of 0.50 PAA/lignin was used to prepare a pretreated lignin for further oxidation with O/A. The pretreated lignin was oxidized with oxygen in alkaline medium under conditions described previously. Alkali was added at a concentration of 0.13N Na_2CO_3 in order to permit comparison of the PAA-pretreated lignin oxidation results (designated OX-PA) with the OX.13 oxidation results.

FINAL pH AND BASE CONSUMPTION

The pH after oxidation and the milliequivalents of acid required to titrate each reacted sample liquor to pH 7.0 for oxidation runs OX.13 and OX-PA are compared in Table XIX.

TABLE XIX

pH AFTER REACTION AND MEQ ACID TO TITRATE TO pH 7.0

Sample Number	Oxidation Run OX.13 pH After Reaction	Oxidation Run OX.13 Meq Acid to Titrate to pH 7.0	Oxidation Run OX-PA pH After Reaction	Oxidation Run OX-PA Meq Acid to Titrate to pH 7.0
SM	10.965	7.51	10.223	6.17
0.0	10.970	7.24	10.325	5.83
0.5	10.079	4.50	9.570	3.30
1.0	9.669	3.83	--	--
2.0	9.486	3.44	9.453	2.48
4.0	9.433	2.74	--	--
8.0	9.322	2.47	9.435	2.45

Whereas the DL used in the OX.13 oxidation series had been isolated by first adjusting the pH to 9.0 prior to freeze-drying, the PAA-treated lignin was isolated directly from acetic acid/water (9/1). Therefore, carboxylic acid groups in the lignin for the latter oxidation series were isolated as the free acid. Thus a part of the alkali charge in the OX-PA oxidation series was effectively neutralized by these acids and the initial pH of the cooking liquor was lower for the OX-PA oxidation than for the OX.13 oxidation.

In spite of the lower initial pH of the OX-PA oxidation, the final pH for both oxidation systems approached the same level, suggesting that a pH of 9.3-9.4 was a lower limit for lignin degradation with O/A. Such an interpretation would be consistent with a mechanism of lignin degradation in which the degradation was initiated by formation of a pH dependent ionic species. The pH range suggests these ionic species were phenolate anions.

Milliequivalents of base consumed for a given time of oxidation was computed by subtracting the milliequivalents of acid to titrate to pH 7.0 for the given time of oxidation from the milliequivalents of acid required

to titrate the SM sample. Base consumption as a function of time of oxidation is plotted in Fig. 32. Both oxidation systems were characterized by rapid initial base consumption followed by a leveling off in base consumption after about 2 hours. As discussed above, the OX-PA samples were initially at a lower pH and therefore had less base available for consumption before reaching the 9.3-9.4 level.

ASH AND MOISTURE CONTENTS

Ash contents of the OX-PA samples were computed according to the procedure described previously. Ash contents are tabulated in Table XLI of Appendix X. Ash contents of the OX-PA samples were approximately 50%. Values for chemical analyses were corrected for ash contents.

Moisture contents, based on o.d. weight, are tabulated in Appendix I. Values for chemical analyses were corrected for moisture content.

METAL CONTENT

Equivalent amounts of lignin, alkali, and water were used in each oxidation run. Any catalysis due to the presence of metal ions should have been comparable in all samples. Therefore, comparison of trends in changes in the chemical properties of the oxidized lignins as a function of time of reaction would be expected to be valid. Metal analyses of selected samples from the OX.13 and the OX-PA series are presented in Table XXXII of Appendix II.

ELEMENTAL ANALYSIS AND METHOXYL CONTENT

Fundamental analytical data for the OX.13 and the OX-PA series are compared in Table XX. There was some scatter in the data for the OX-PA run,

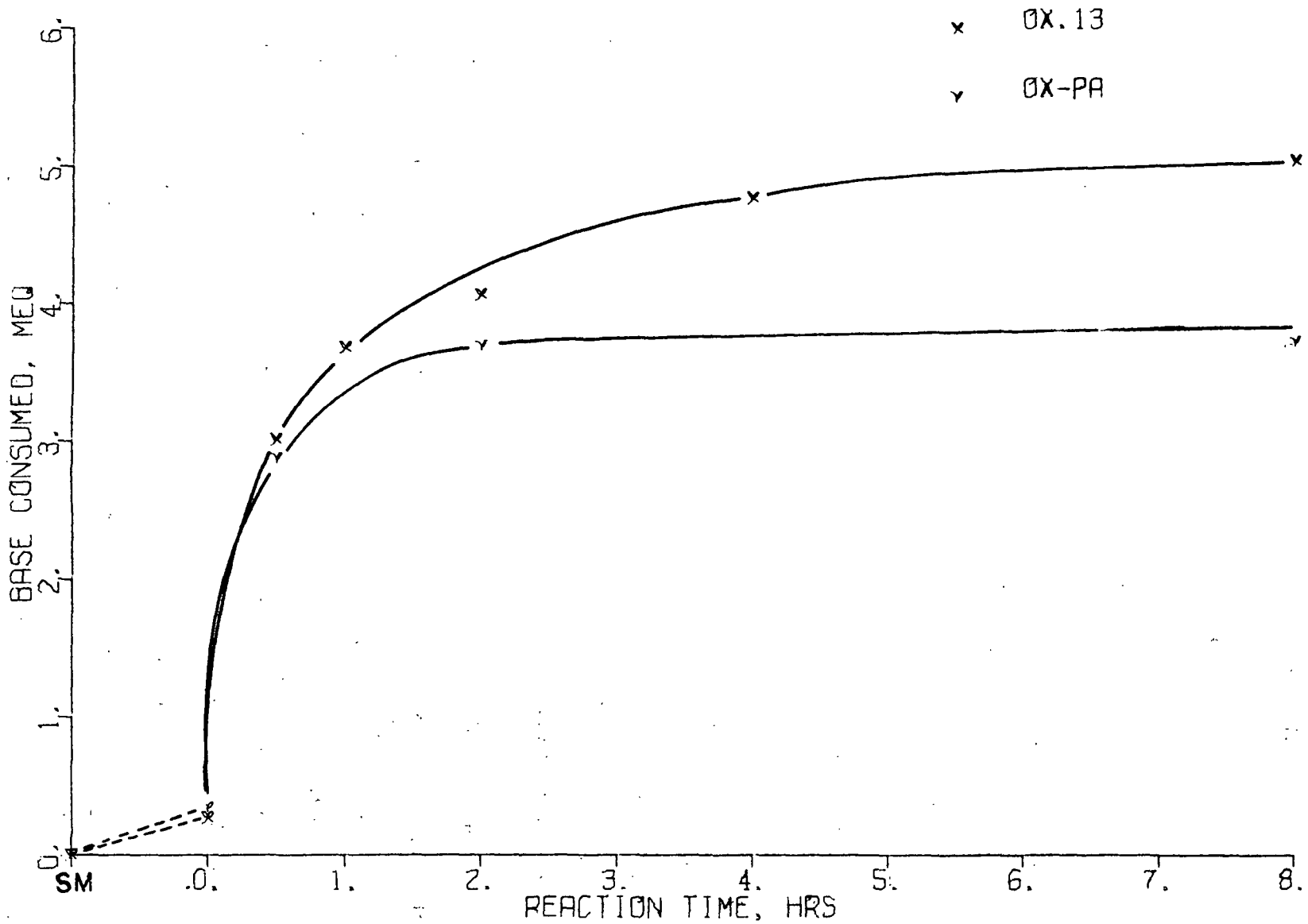


Figure 32. Base Consumption vs. Reaction Time for OX.13 and OX-PA Series

TABLE XX

METHOXYL AND ELEMENTAL ANALYSES FOR OX.13 AND OX-PA SAMPLES

Sample Number	Elemental Analysis[a,b] C, %	H, %	O, %	OCH_3, %	Elemental Analysis Adjusted for Methoxyl C, %	H, %	O, %
OX.13-SM	64.57	5.97	29.46	15.65	58.51	4.45	21.39
-0.0	59.15	6.21	34.64	14.83	53.41	4.77	25.99
-0.5	Not determined			13.47			
-1.0	59.36	5.40	35.24	13.07	54.30	4.13	28.50
-2.0	56.39	4.88	38.73	12.75	51.46	3.64	32.16
-4.0	51.51	4.34	44.15	11.31	47.13	3.24	38.32
-8.0	51.91	4.51	43.58	9.49	48.24	3.59	38.69
OX-PA-SM	Not determined			13.62			
-0.0	61.85	5.13	33.02	14.39	56.28	3.73	25.61
-0.5	55.27	5.39	39.34	12.00	50.63	4.22	33.16
-2.0	57.69	5.45	36.86	10.64	53.57	4.41	31.37
-8.0	56.70	5.07	38.23	9.60	52.98	4.13	33.28

[a]Based on moisture-free (dried over P_2O_5 under vacuum) weight, corrected for ash.

[b]Average of duplicate determinations.

probably associated with the high ash content of these samples. Oxygen content computed by difference from carbon-hydrogen analyses was higher in the OX.13 samples than in the corresponding OX-PA samples, suggesting more extensive oxidation of the OX.13 samples. On the other hand, it appeared that methoxyl contents of the OX-PA samples were somewhat lower than the corresponding samples in the OX.13 series, indicating that the OX-PA series had undergone more extensive demethylation. The methoxyl content of the OX-PA-SM sample appeared to be significantly lower than the unmodified OX.13 sample. However, the 0.0-hour samples for both series were essentially equivalent. Both analyses were run in triplicate. It was not possible to say which of the two values represented a bad sample.

ULTRAVIOLET SPECTROSCOPY

Direct Ultraviolet Spectroscopy

The direct UV spectra of the oxidized lignins from the OX-PA series are compared in Fig. 33. The direct UV spectra of the OX.13 series were compared in Fig. 16. All samples were adjusted to pH 9.0 prior to freeze-drying.

As was observed with the OX.13 samples, there was a general increase in absorptivity at about 260 and 300 nm with the absorptivity at 280 nm remaining relatively constant with increasing time of oxidation. Analysis of the oxidized lignins indicated that methoxyl and phenolic hydroxyl contents of these samples decreased with increasing time of reaction. Since both of these functionalities contribute to the absorption band at 280 nm, it would be expected that this band should decrease in intensity. As was discussed in the previous section comparing UV spectra of the OX.13 and OX.50 samples, the increased absorptivity at 260 and 300 nm and the lack of a decrease in absorptivity at 280 nm may be interpreted to suggest formation of biphenyl linkages in the lignin as a consequence of charging the reactor with oxygen and alkali.

An attempt to confirm this interpretation was made by extending the flat portion of the curve between 300 and 320 nm to establish a base line for measuring the height of the 280 nm peak in each sample. Results from this analysis are summarized in Table XXI.

The results in Table XXI illustrate that, relative to any change in absorptivity between 300 and 320 nm, the intensity of the absorptivity at 280 decreased with increasing time of oxidation, as would be expected from loss of methoxyl and phenolic hydroxyl. These results confirm the interpretation

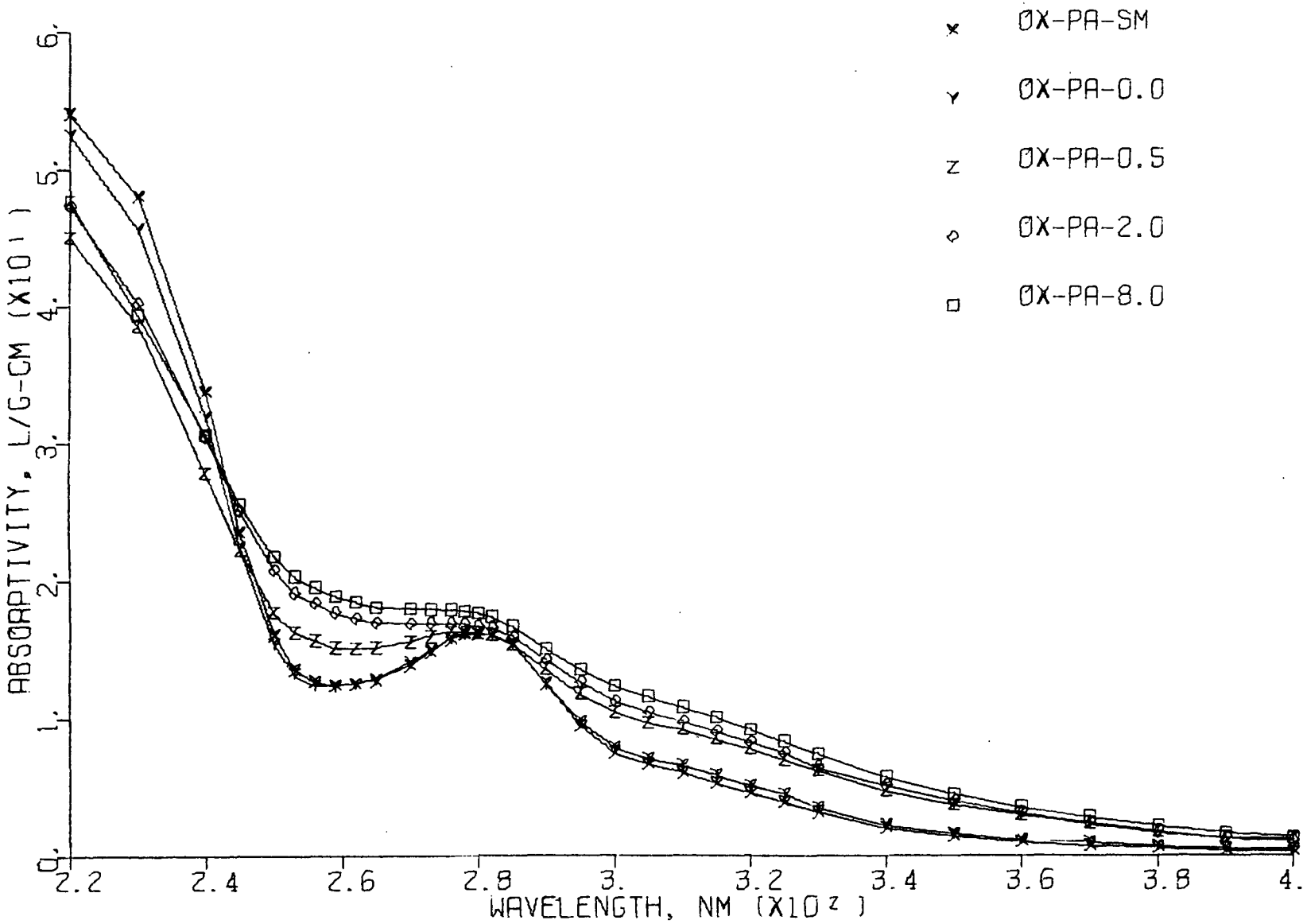


Figure 33. Comparison of Direct Ultraviolet Spectra of OX-PA Reaction Samples

that there were concurrent increases in absorptivity at 260, 280 and 300 nm, thus supporting the hypothesis that the increased absorptivity was related to formation of biphenyl structures with time of oxidation.

TABLE XXI

PEAK HEIGHTS AT 280 NM IN DIRECT ULTRAVIOLET SPECTRA[a]

Sample Number	Oxidation Run OX.13 Absorptivity, 1/g-cm	Oxidation Run OX-PA Absorptivity, 1/g-cm
SM	4.91	5.66
0.0	3.92	5.46
0.5	3.85	3.13
1.0	3.37	--
2.0	1.72	2.49
4.0	1.12	--
8.0	1.08	2.08

[a]The base line for determination of peak height was established by extending a line through absorptivities at 300 and 320 nm as shown in Fig. 18.

The direct UV spectra of the SM and 8.0-hour samples from the two oxidation series are compared in Fig. 34 and 35, respectively. Absorptivity of the OX-PA-SM sample was lower than the corresponding OX.13-SM sample at all wavelengths. However, in the highly oxidized samples (8.0 hr) the UV spectra were identical within experimental accuracy, indicating comparable levels of degradation.

Ultraviolet Difference Spectra

The UV difference spectra of the OX-PA samples are compared in Fig. 36. The UV difference spectra of the OX.13 samples were compared in Fig. 21. As before, the predominant change in the UV difference spectra was the disappearance of the major absorption bands at 250 and 300 nm with time of oxidation.

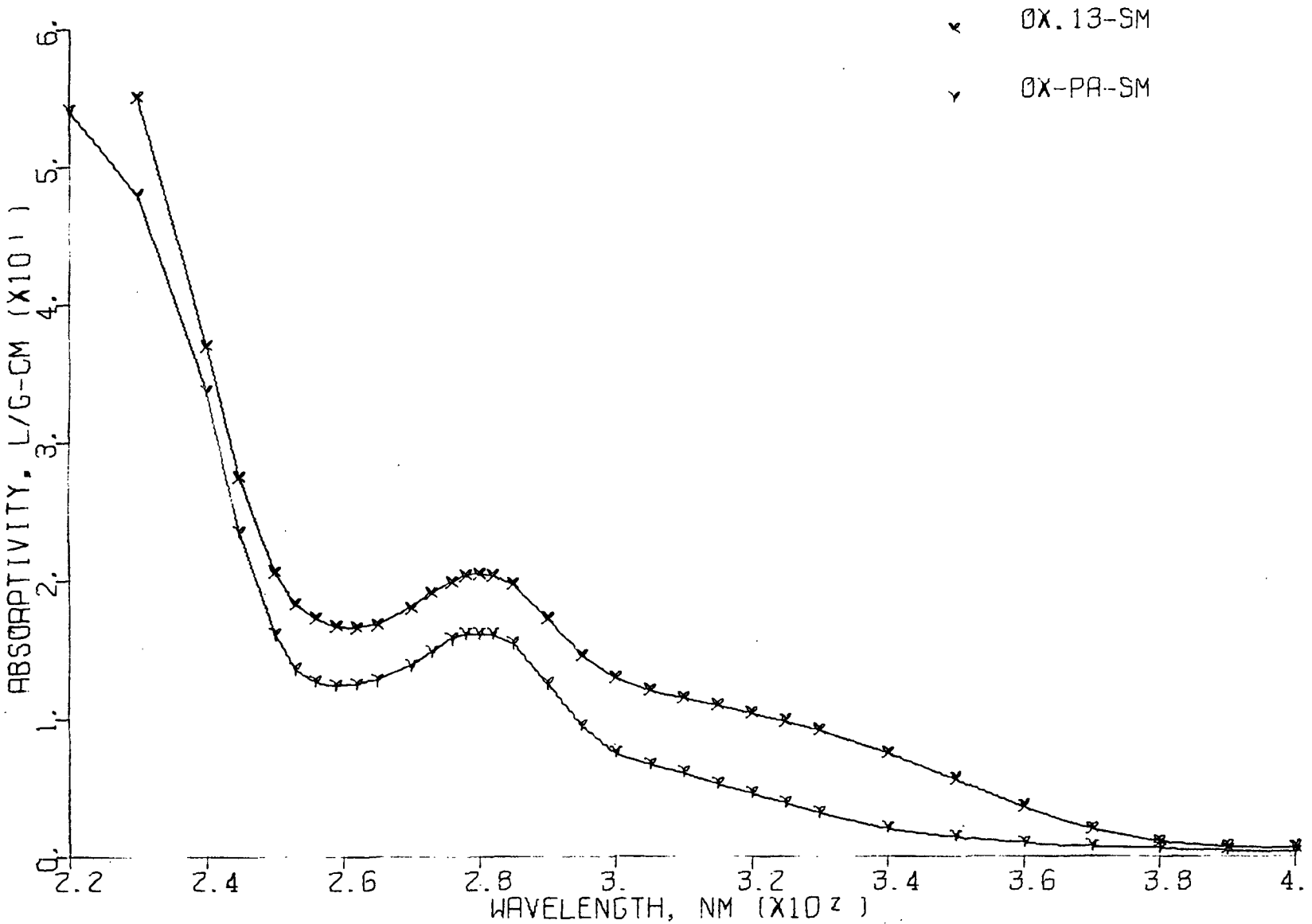


Figure 34. Comparison of Direct Ultraviolet Spectra of OX.13-SM and OX-PA-SM Samples

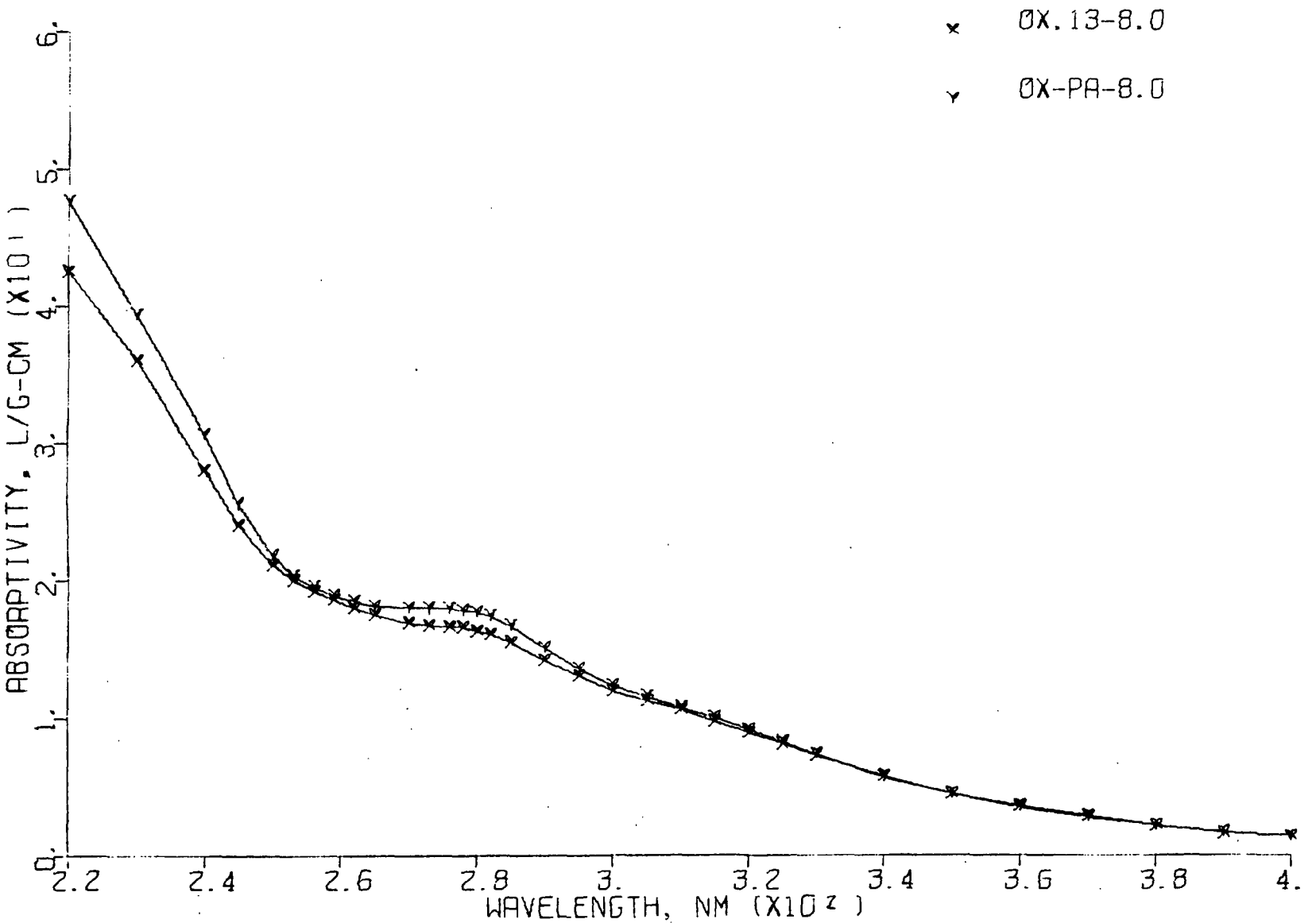


Figure 35. Comparison of Direct Ultraviolet Spectra of OX.13-8.0 and OX-PA-8.0 Samples

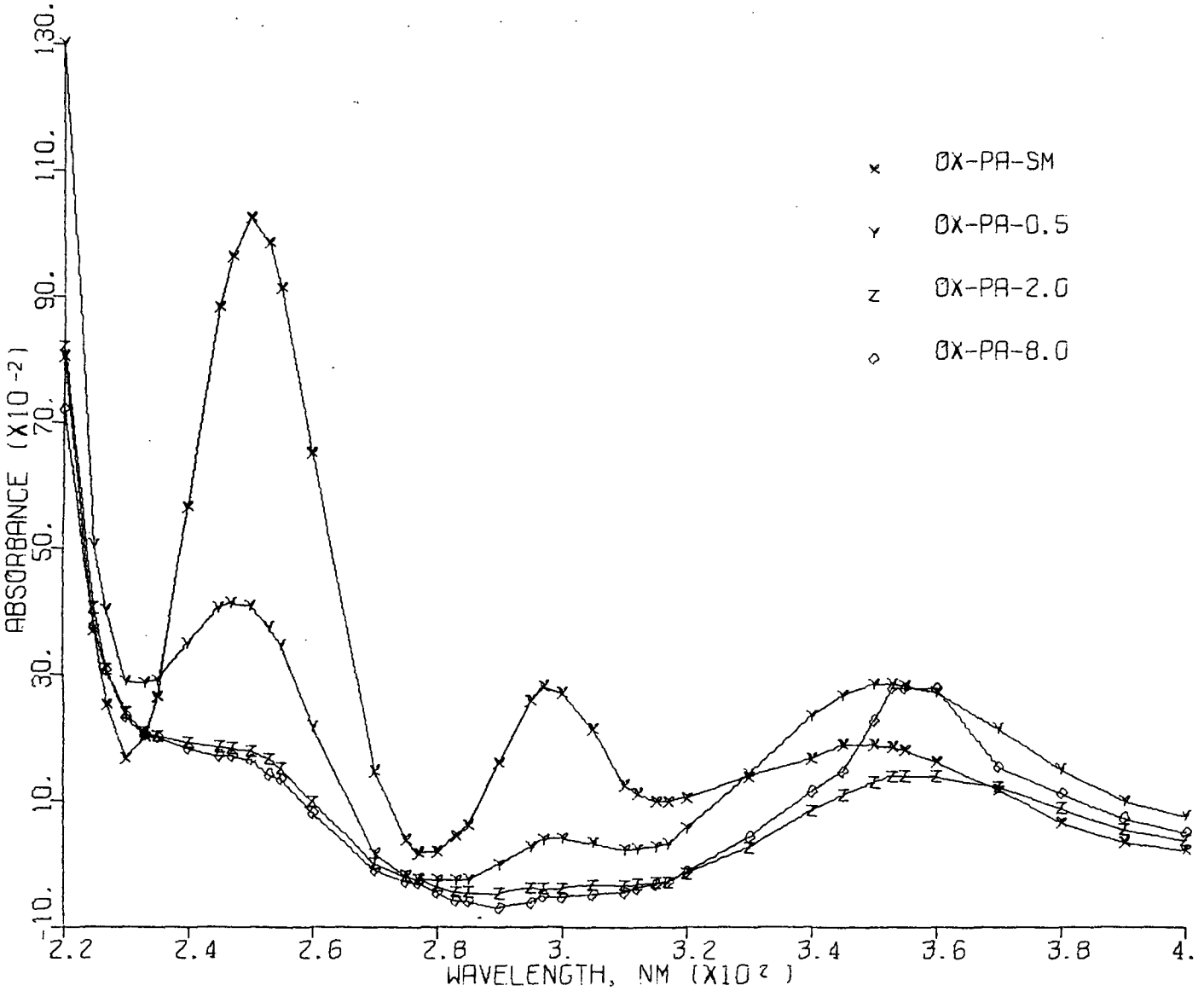


Figure 36. Comparison of Ultraviolet Difference Spectra of OX-PA Samples

Free phenolic hydroxyl contents are evaluated quantitatively from the intensity of the 250 nm band of the UV difference spectra in a subsequent section.

The UV difference spectra of the OX.13-SM and OX-PA-SM samples are compared in Fig. 37. The major difference between these spectra was a shift in the maximum at 360 nm in the OX.13-SM sample to about 345 nm in the OX-PA-SM sample. Absorbance in this region has been assigned to π-systems conjugated to an aromatic ring containing an ionized phenolic hydroxyl group. Carbon-13 NMR spectra of the respective starting materials (discussed subsequently) indicated that a major effect of the PAA pretreatment was the destruction of α-carbonyls in the lignin. The shift in the UV difference spectra from 360 to 345 nm may well be related to the loss of α-carbonyls.

INFRARED SPECTROSCOPY

Infrared spectra of the OX-PA samples are compared in Fig. 38. Infrared spectra of the OX.13 samples were compared in Fig. 23. Samples were isolated at pH 9 and thus, carboxylic acids were present as the carboxylate anion. Changes in the IR spectra of the OX-PA samples were the same as discussed previously for the OX.13 samples, indicating principally formation of carboxylate groups and loss of aromaticity as a function of time of oxidation.

Infrared spectra of the OX-PA-SM and OX.13-SM samples are compared in Fig. 39. Increased absorbance of the bands near 1600 and 1400 cm^{-1} in the OX-PA-SM sample IR relative to the OX.13-SM IR indicated that formation of carboxylate groups was a major consequence of the PAA pretreatment.

CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Assignments for chemical shifts in the ^{13}C NMR spectrum of the DL have been discussed previously (Table VI). The ^{13}C NMR spectrum of the OX-PA-SM

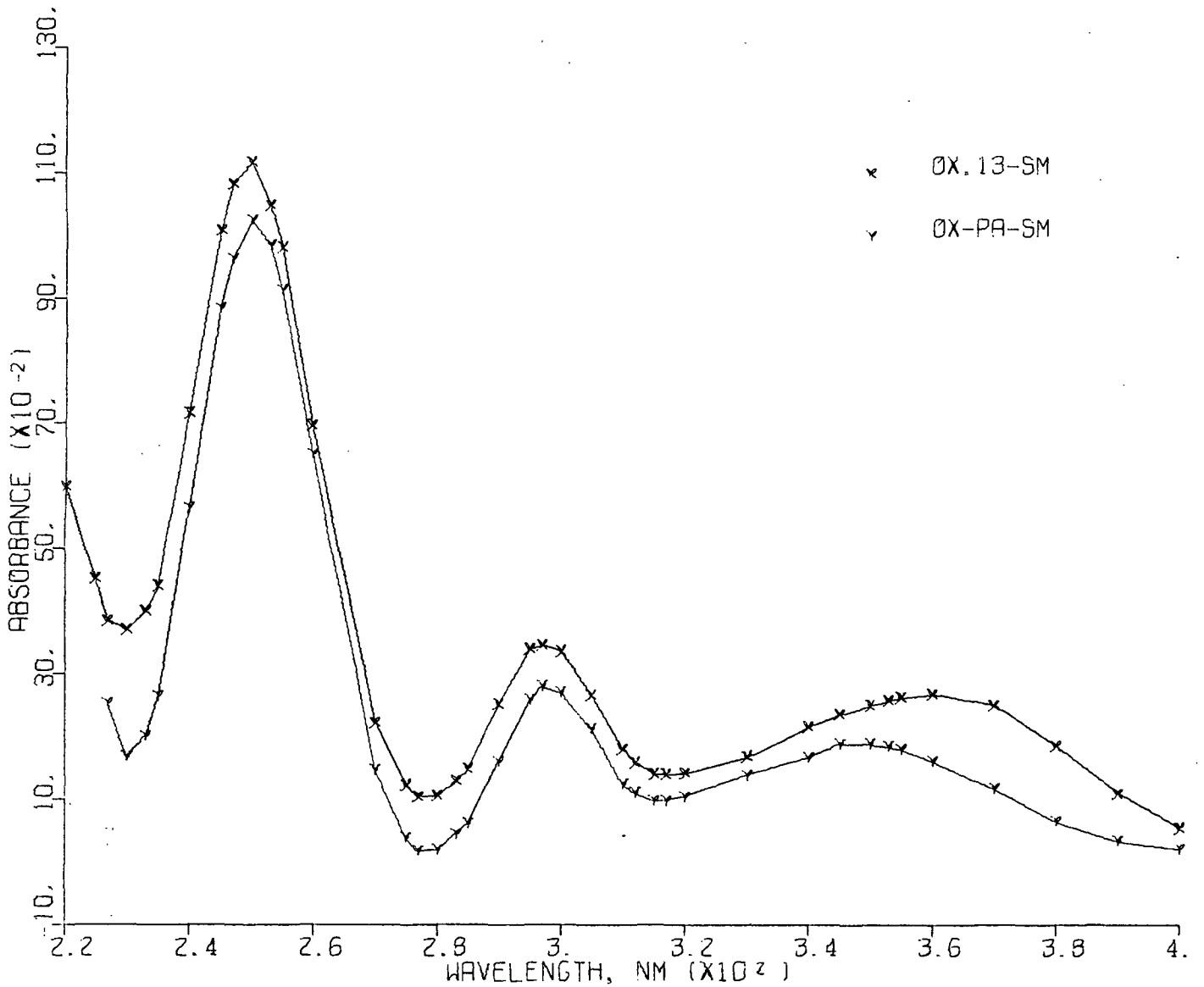


Figure 37. Comparison of Ultraviolet Difference Spectra of OX.13-SM and OX-PA-SM Samples

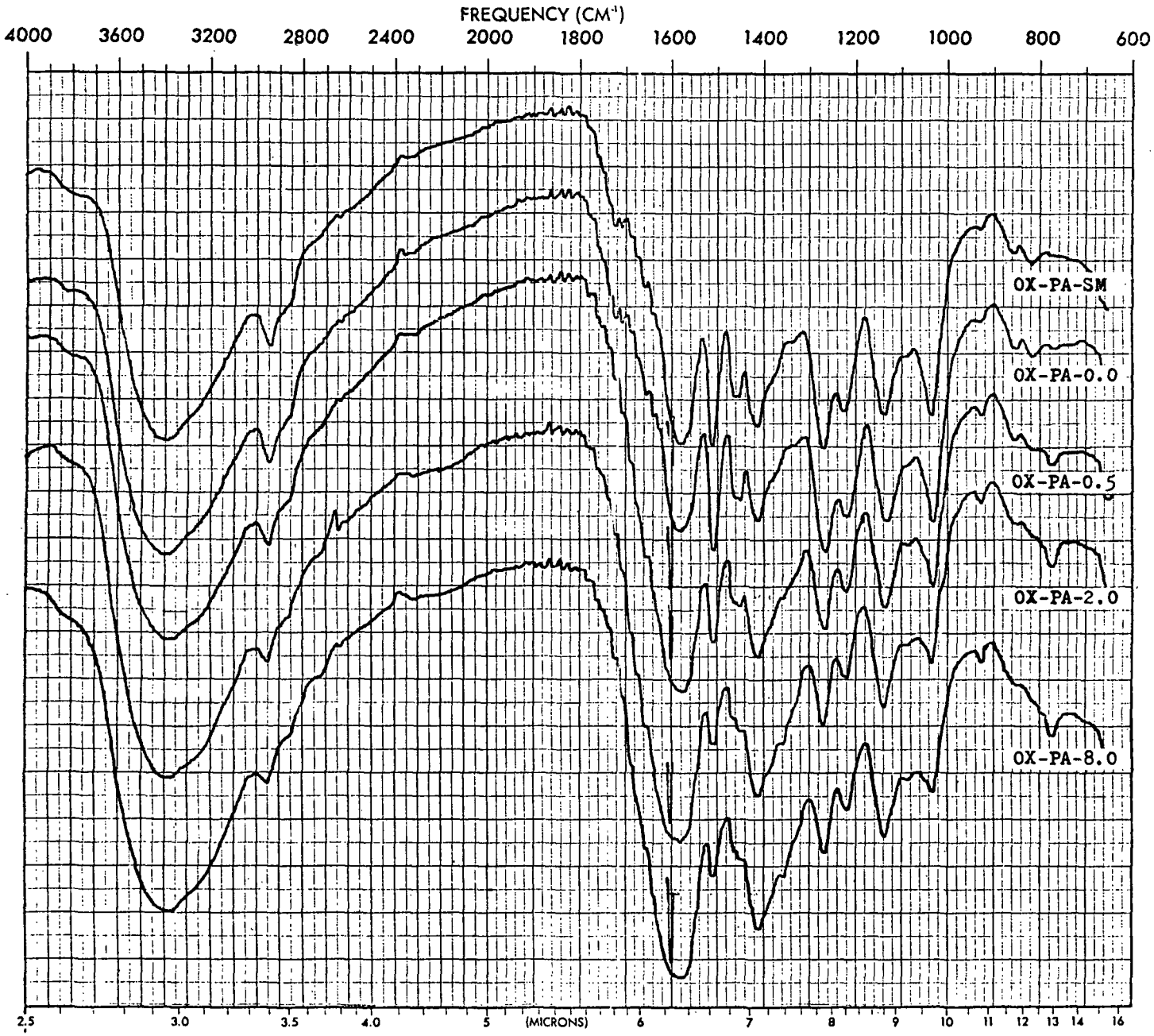


Figure 38. Comparison of Infrared Spectra of OX-PA Reaction Samples

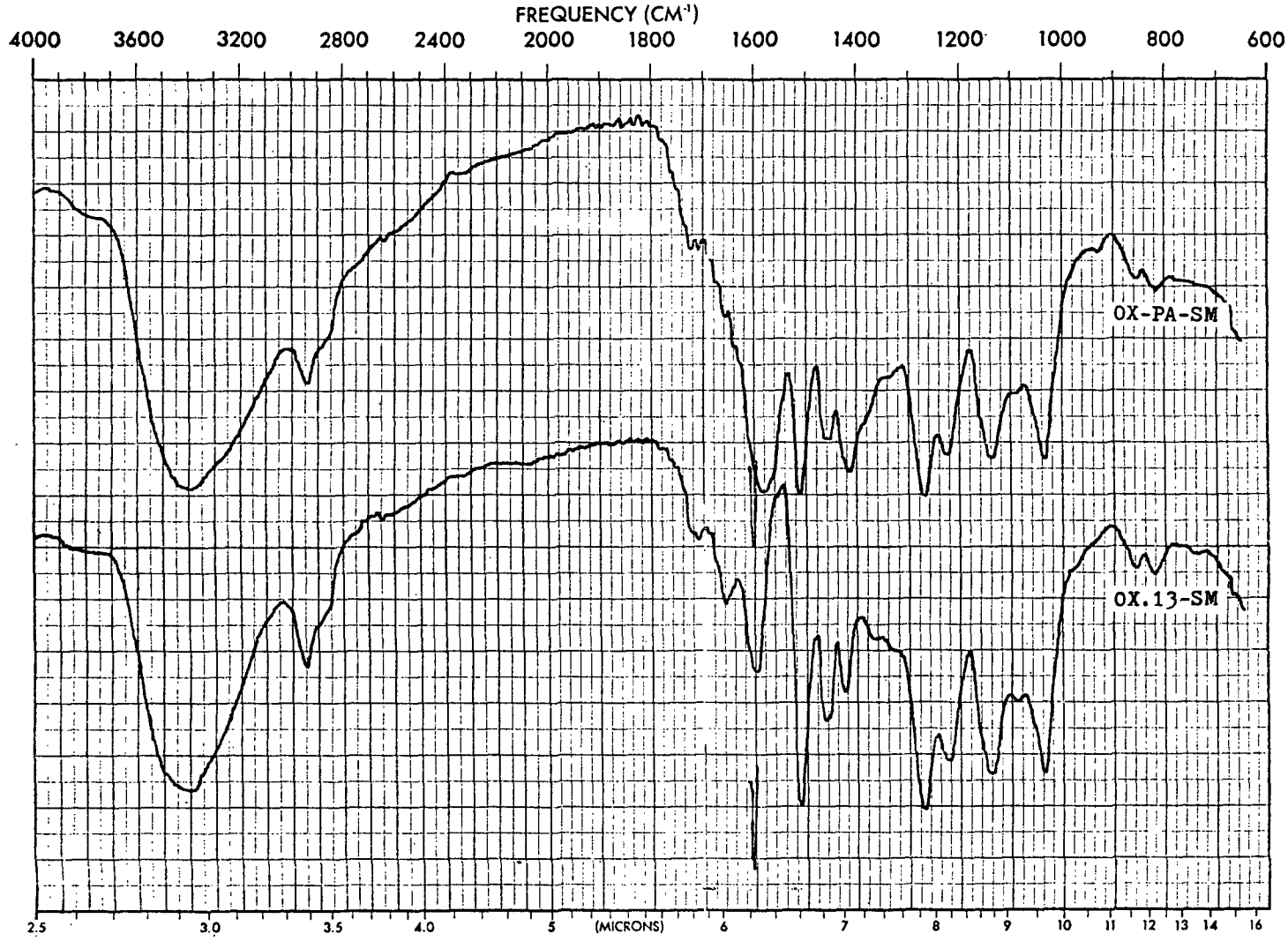


Figure 39. Comparison of Infrared Spectra of OX-PA-SM and OX.13-SM Samples

sample is presented in Fig. 40. The ^{13}C NMR spectrum of the DL was shown in Fig. 12. Chemical shifts of the numbered signals in the OX-PA-SM spectrum are compared with the corresponding values from the DL spectrum in Table XXII.

TABLE XXII

CHEMICAL SHIFTS IN THE ^{13}C NMR SPECTRA OF LOBLOLLY PINE DIOXANE LIGNIN AND PEROXYACETIC ACID MODIFIED LIGNIN

Signal Number	δ-Value in ppm[a,b,c]	
	Loblolly Pine DL	PAA Modified DL
1	194.6 w	194.6 w
2	191.4 w	191.6 w
3a	--	173.8 w
3b	--	172.8 s
3c	--	167.8 w
3d	--	162.8 w
3e	157.8 w	157.5 w
4	154.7 w	--
6	152.7 w	152.8 w
7	150.4 s	150.3 s
8	147.7 s	147.7 s
9	146.1 m	146.1 m
10	144.8 w	144.5 w
11	138.7 vw	138.9 vw
12	136.2 m	135.9 m
13	133.3 m	133.3 m
14	131.8 w	131.7 w
15	130.3 w	130.7 w
16	129.2 m	129.2 m
17	126.8 w	--
18	120.1 s	120.1 s
19	117.4 m	117.3 m
20	115.4 s	115.4 s
20a	--	115.0 s
21	113.0 w	113.5 w
22	112.0 s	111.9 s
22a	111.2 s	111.2 s
25a	93.8 w	93.5 w
25	88.0 m	88.2 m
27	85.9 m	86.3 m
28	85.0 m	85.3 m
29	82.9 w	83.0 w
31	73.0 s	73.0 s
31a	--	68.2 m

See end of table for footnotes.

TABLE XXII (Continued)

CHEMICAL SHIFTS IN THE ^{13}C NMR SPECTRA OF LOBLOLLY PINE DIOXANE LIGNIN AND PEROXYACETIC ACID MODIFIED LIGNIN

Signal Number	δ-Value in ppm[a,b,c]	
	Loblolly Pine DL	PAA Modified DL
32	64.1 m	64.1 m
33	61.2 s	61.2 s
34	56.2 vs	56.2 vs
35	54.6 w	54.5 w
36	42-45 vw	42-45 vw
36a	39.4 w	39.4 w
36b	--	23.0 w
36c	--	20.7 s
36d	41.0 w	--
36e	35.1 vw	--

[a] δ-Values in ppm from TMS; spectra run in deuteroacetone/deuterium oxide (9/1, v/v).

[b] Intensities: w = weak, m = medium, s = strong, vw = very weak, vs = very strong.

[c] Assignments of signals are outlined in Table VI.

Comparison of the ^{13}C NMR spectra of the two samples indicated several changes had occurred in the OX-PA-SM sample as a result of the PAA pretreatment:

1. signal 1 was significantly reduced in intensity in the OX-PA-SM sample, assigned to γ-CO in cinnamaldehyde and α-CO;
2. signals 3b and 36c appeared in the OX-PA-SM sample; these were assigned to acetic acid not removed by freeze-drying the PAA modified lignin;
3. signals 3a, 3c, 3d, all very weak, were in the general spectral region assigned to carboxylic acids and were not observed in the DL spectrum (100). These signals tend to be weak in general due to the long relaxation times;
4. signal 4 disappeared in the OX-PA-SM sample; assigned to C-4 in guaiacyl with α-CO, etherified;

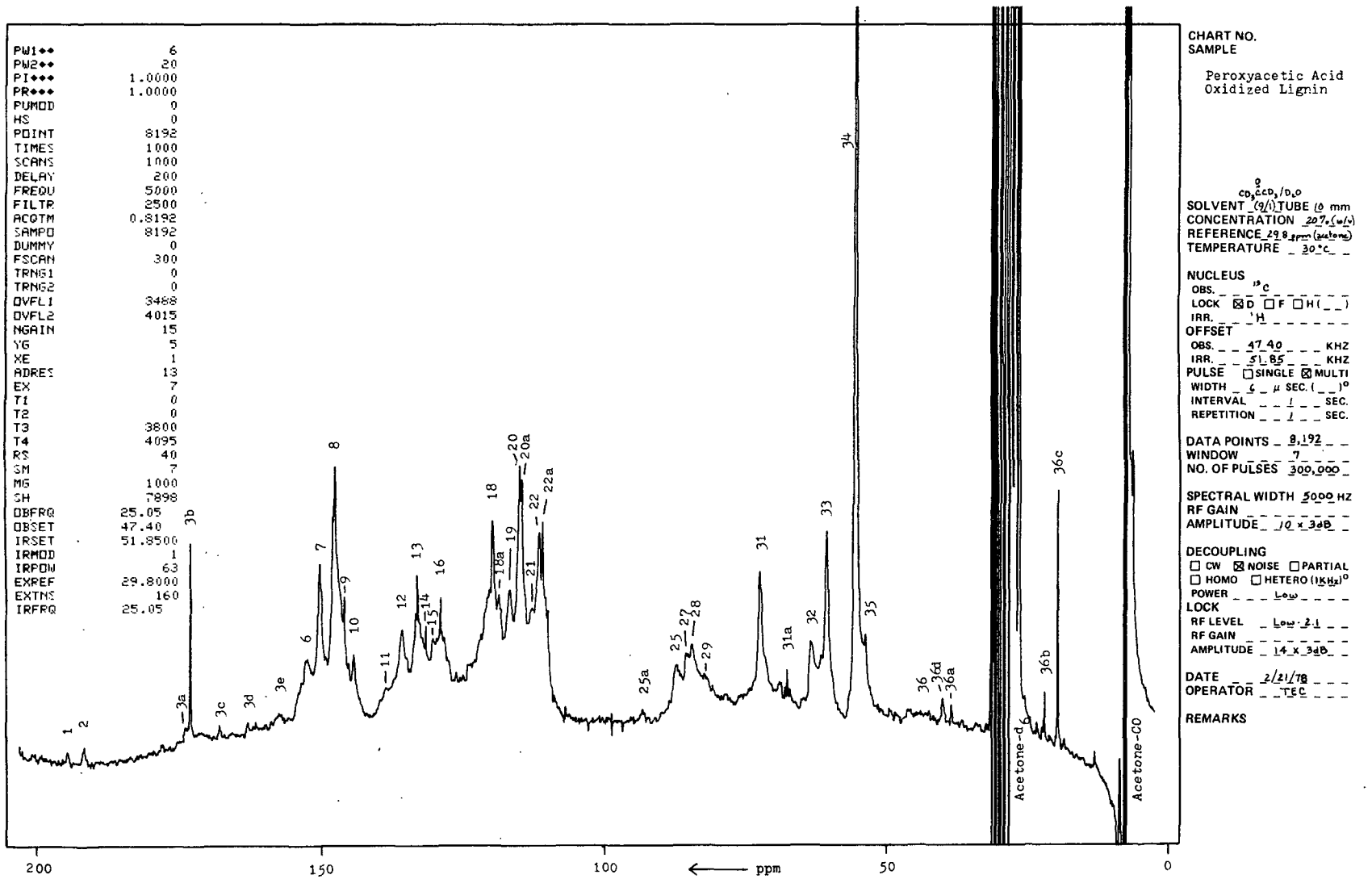


Figure 40. ^{13}C NMR Spectrum of OX-PA-SM Sample

5. signal 17 disappeared in the OX-PA-SM sample; assigned to C-6 in guaiacyl with α-CO, etherified; and
6. signal 36b appeared in the OX-PA-SM sample; not assigned.

The reduction in the intensity of signal 1, together with the disappearance of signals 4 and 17 in the OX-PA-SM spectrum, indicated that a major consequence of the PAA pretreatment was the destruction of α-carbonyl groups. Interestingly, there were several other signals which have been assigned to α-carbonyls which appeared to be unchanged by the PAA pretreatment:

1. signal 6; assigned to C-4 in guaiacyl with an α-CO and a free phenolic, or an α-CHO, etherified;
2. signal 7; assigned to C-4 in guaiacyl, etherified or C-3 in guaiacyl with an α-CO, etherified; and
3. signals 28 and 29; assigned to C-β in β-aryl ethers with an α-CO.

Both signals 6 and 7 have double assignments. The results suggest that the α-carbonyl contributes much less to the respective signal than the alternative assignment. Both signals 28 and 29 were broad and fairly weak, making detection of changes in these signals difficult. An alternative explanation would be that the presence of a β-aryl ether inhibits reaction of the α-carbonyl.

PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Integrals of the PMR spectra of the OX-PA samples are compared in Table XXIII. The integrals of the PMR spectra of the OX.13 samples were presented in Table XII.

Ignoring the OX-PA-0.5 sample, which appeared to be anomalous, there were several apparent trends in the integrals with increasing time of oxidation: decreases in the H_a and H_{PhOAc} integrals and an increase in the H_α integral.

Comparison with the PMR integrals for the OX.13 series did not appear to provide any useful results. The solubility problems associated with preparation of the acetylated derivatives, discussed for the OX.13 and OX.50 samples, were likewise observed in the preparation of the OX-PA samples. These problems leave the interpretation of the PMR data open to question.

TABLE XXIII

INTEGRALS OF RANGES FOR CHEMICAL SHIFTS FOR PROTONS IN ACETYLATED OX-PA SERIES LIGNINS

Range[a]	Symbol	Total Integral, %				
		SM	0.0	0.5	2.0	8.0
1	H_a	16.9	16.5	18.4	15.7	15.8
2	H_α	6.2	6.1	4.5	9.4	9.8
3	H_{OCH_3}[b]	39.1	22.3	17.3	15.4	15.2
	$H_{\beta\gamma}$		17.1	20.2	20.3	21.0
4	$H_{\alpha'}$	4.4	5.2	4.6	5.2	5.9
5	H_{PhOAc}	7.0	6.6	7.7	5.1	4.7
6	H_{AlOAc}	21.8	20.8	22.7	24.0	22.3
7	$H_{\beta'\gamma'}$	4.6	5.4	4.6	4.8	5.2

[a] Shift ranges and assignments are listed in Table VI.

[b] H_{OCH_3} and $H_{\beta\gamma}$ were calculated from methoxyl contents and elemental analyses in Table XX.

PHENOLIC HYDROXYL

One objective of the PAA pretreatment of the lignin was to increase the phenolic hydroxyl content of the lignin. Phenolic hydroxyl contents determined from UV difference spectra by the method of Wexler (83) for the OX.13 and the OX-PA series are compared in Table XXIV.

TABLE XXIV

FREE PHENOLIC HYDROXYL CONTENTS OF OXIDIZED LIGNINS CALCULATED FROM UV DIFFERENCE SPECTRA

Sample Number	Oxidation Run OX.13[a] Phenolic Hydroxyl, %	Oxidation Run OX-PA[b] Phenolic Hydroxyl, %
SM	2.42	2.52
0.0	1.97	2.26
0.5	0.79	0.68
1.0	0.19	--
2.0	0.24	0.13
4.0	0.00	--
8.0	0.11	0.09

[a]Calculated from UV difference spectra in Fig. 21.

[b]Calculated from UV difference spectra in Fig. 36.

Phenolic hydroxyl contents computed from the integrals of the acetylated PMR spectra according to the procedure of Morohoshi and Sakakibara (79) are compared in Table XXV.

TABLE XXV

FREE PHENOLIC HYDROXYL CONTENTS OF OXIDIZED LIGNINS CALCULATED FROM THE INTEGRALS OF PMR SPECTRA

Sample Number	Oxidation Run OX.13[a] Phenolic Hydroxyl, %	Oxidation Run OX-PA[b] Phenolic Hydroxyl, %
SM	3.0	--
0.0	2.8	2.3
0.5	--	2.9
1.0	2.3	--
2.0	2.3	1.9
4.0	1.6	--
8.0	1.2	1.6

[a]Calculated from PMR integrals in Table XII and from elemental analyses in Table XX.

[b]Calculated from PMR integrals in Table XXIII and from elemental analyses in Table XX.

Phenolic hydroxyl contents calculated from UV difference spectra indicated that, for both the OX.13 and the OX-PA oxidation series, the phenolic hydroxyl content decreased rapidly in the early stages of the oxidation. As was noted previously, the absolute values calculated from UV difference spectra may be somewhat open to question, as the empirical factor computed by Wexler (83) was based on nonoxidized lignin model compounds. However, the virtual disappearance of the strong absorption bands at 250 and 300 nm in the UV difference spectra lends strong qualitative support to the disappearance of phenolic hydroxyls.

The PAA pretreatment of the DL resulted in only a modest increase in phenolic hydroxyl, as evidenced by the increase from 2.42 to 2.52%. The lack of a more significant increase in phenolic hydroxyl due to the PAA pretreatment is probably a reflection of the greater reactivity of the hydroxylated intermediates, relative to the starting material.

Phenolic hydroxyl calculated from the integrals of the PMR spectra of the acetylated samples likewise showed a decrease in phenolic hydroxyl with increasing time of oxidation. However, levels of phenolic hydroxyl calculated from the integrals of the PMR spectra were considerably higher than levels calculated for the corresponding samples from UV difference spectra. As has been discussed, the discrepancy between phenolic hydroxyl contents calculated by the two methods is thought to arise from a differential solubility of the acetylated lignins. Thus, the portion of the oxidized lignin containing a relatively high phenyl acetoxy content appears to have been preferentially dissolved in the deuterated chloroform.

CARBONYL

Carbonyl contents of the OX.13 and OX-PA samples, determined by oximation (86), are compared in Table XXVI. Both oxidation series were characterized by a rapid decrease in carbonyl in the early stages of reaction. It was noted previously that, based on ^{13}C NMR, a major consequence of the PAA pretreatment was loss of α-carbonyl groups. Adler and Marton (85) estimated that approximately one-third of the carbonyls in MWL were present as α-carbonyls. Comparison of the OX.13-SM and OX-PA-SM samples indicated, however, that there were equivalent levels of carbonyl in the two samples, indicating the formation of carbonyl groups as a result of the PAA treatment. Based on the model compound studies of Farrand (19,98), one can propose that the lack of a decrease in the carbonyl content of the OX-PA-SM sample indicates formation of quinone structures.

TABLE XXVI

CARBONYL CONTENTS OF OXIDIZED LIGNINS

Sample Number	Oxidation Run OX.13 Carbonyl, %	Oxidation Run OX-PA Carbonyl, %
SM	2.63	2.54
0.0	2.06	--
0.5	0.40	0.97
1.0	0.17	--
2.0	0.74	0.00
4.0	0.40	--
8.0	0.00	--

CARBOXYL

Carboxyl contents of the OX.13 and OX-PA samples, estimated by potentiometric titration, are compared in Table XXVII. Both oxidation series were

characterized by a rapid increase in carboxyl during the initial reaction. The OX-PA series, however, had a much higher initial carboxyl content as a result of the PAA pretreatment. Whereas the OX-PA series appeared to have reached a maximum carboxyl content after only 2 hours of reaction, the OX.13 series carboxyl content continued to increase throughout the oxidation, approaching the same level as the OX-PA sample after 8.0 hours.

TABLE XXVII

CARBOXYL CONTENTS OF OXIDIZED LIGNINS

Sample Number	Oxidation Run OX.13 Carboxyl, %	Oxidation Run OX-PA Carboxyl, %
SM	0.81	7.38
0.0	0.46	6.60
0.5	4.6	13.0
1.0	7.7	--
2.0	9.5	16.0
4.0	10.9	--
8.0	14.5	16.4

MOLECULAR WEIGHT DISTRIBUTION

Studies on the molecular weight distributions of oxidized lignins have been reviewed previously in this thesis. The molecular weight distributions of the OX-PA samples are compared in Fig. 41, determined on Sephadex G-75. As was observed in previous oxidations in this thesis, the elution curves were bimodal, exhibiting both a high and a low molecular weight fraction in the oxidized samples with a general decrease in the absorbance of materials in the intermediate molecular weight range. These results indicate that there was extensive cross-linking of the lignin in the initial oxidation period, followed by degradation of the high molecular weight material to low molecular weight degradation products.

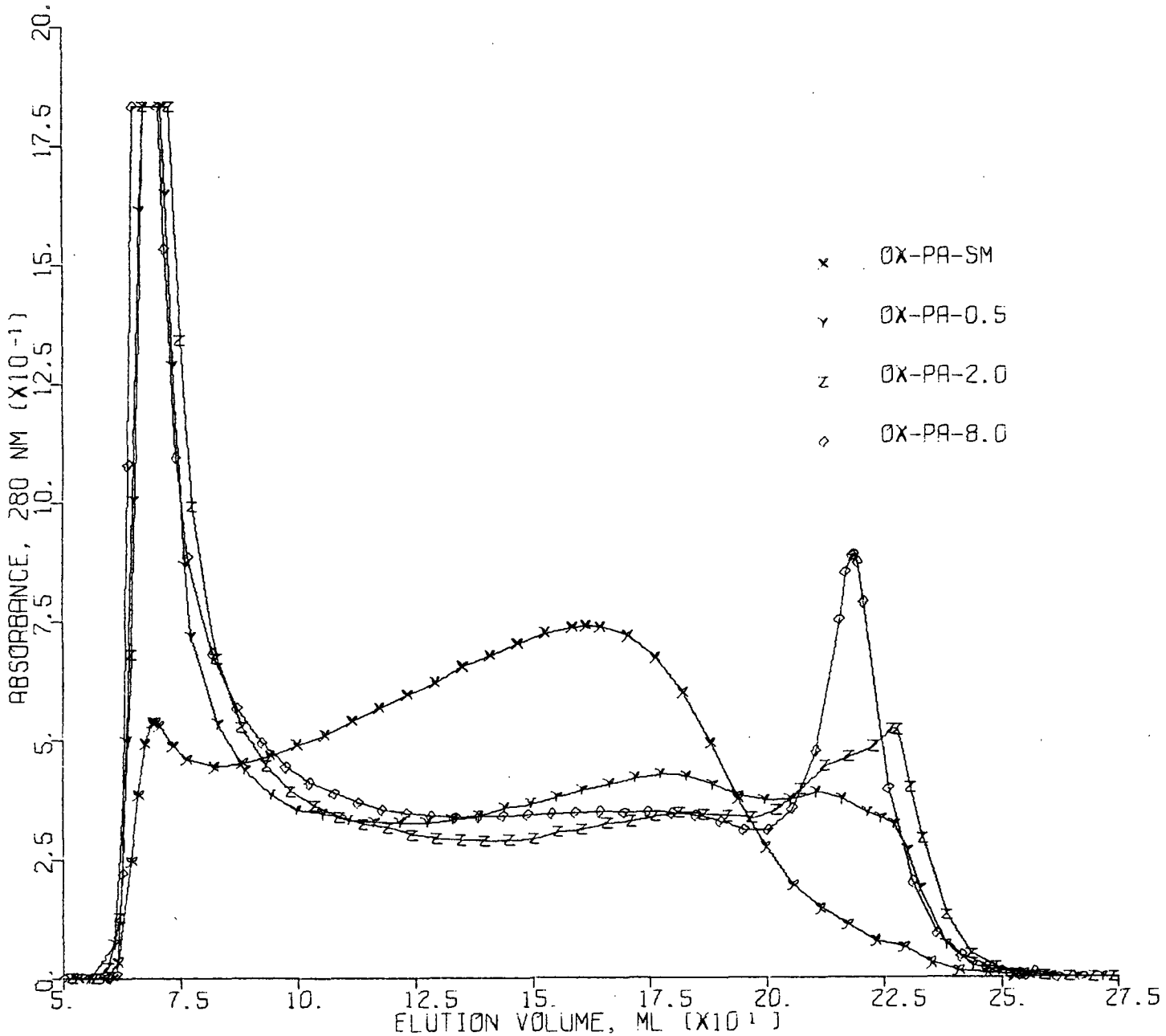


Figure 41. Comparison of Molecular Weight Distributions of OX-PA Reaction Samples Determined by Chromatography on a Column of Sephadex G-75, with Formamide as Solvent

The molecular weight distributions of the OX.13-SM and OX-PA-SM samples are compared in Fig. 42, determined on Sephadex G-75. In general, absorbance of the PAA-modified lignin was lower throughout the distribution. This result was consistent with the lower absorptivity at 280 nm for the PAA-modified lignin relative to the OX.13-SM sample observed in the direct UV spectra. Additionally, the low molecular weight region of the PAA-modified lignin indicated that there was an increase in low molecular weight products due to the PAA pretreatment. In view of carbonyl and carboxyl analyses discussed previously, it may be hypothesized that the PAA pretreatment led to oxidation of α-carbonyl groups to α-carboxylic acids with side-chain cleavage and formation of lower molecular weight material.

The molecular weight distributions of the OX.13-8.0 and OX-PA-8.0 samples are compared in Fig. 43. The larger high molecular weight fraction in the OX-PA-8.0 sample, relative to the OX.13-8.0 sample, is thought to have been a consequence of more extensive cross-linking of the PAA-modified lignin on charging the reactor with oxygen and alkali. One mechanism which may be proposed to account for the more extensive cross-linking is that the oxidation of α-carbonyls to α-carboxylic acids with concurrent lowering of the molecular weight led to greater mobility of the lignin fragments. This greater mobility in turn provided increased access of resonance stabilized radicals for coupling.

SUMMARY — OXIDATION OF DIOXANE LIGNIN WITH OXYGEN/ALKALI — COMPARATIVE STUDIES ON A PEROXYACETIC ACID-MODIFIED LIGNIN

Major changes resulting from the chemical treatment of DL with PAA appeared to be destruction of α-carbonyls, formation of carboxylic acids, and some formation of lower molecular weight products. Only a minor increase (ca. 5%) in phenolic hydroxyl was observed.

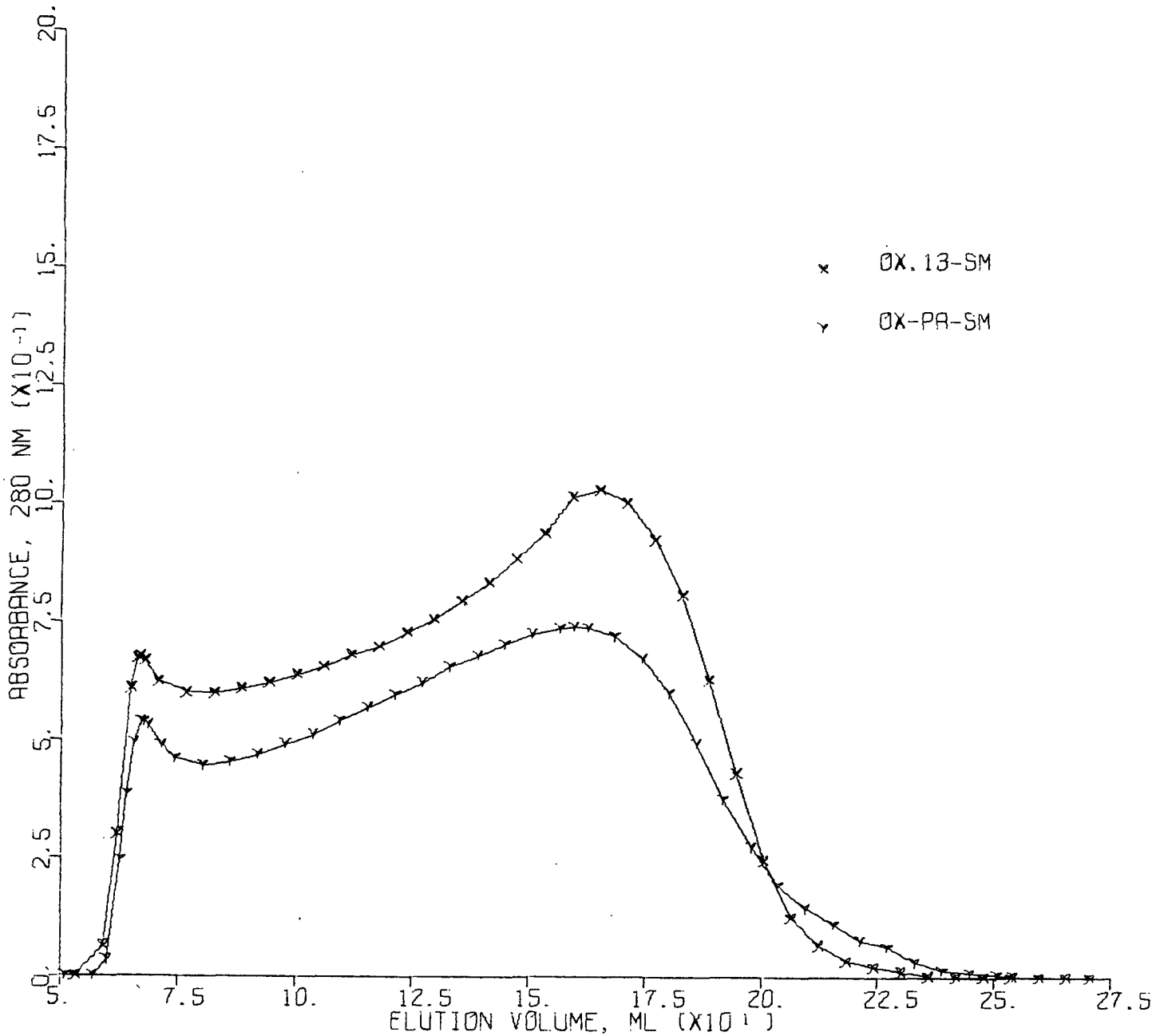


Figure 42. Comparison of Molecular Weight Distributions of OX.13-SM and OX-PA-SM Samples Determined by Chromatography on a Column of Sephadex G-75, with Formamide as Solvent

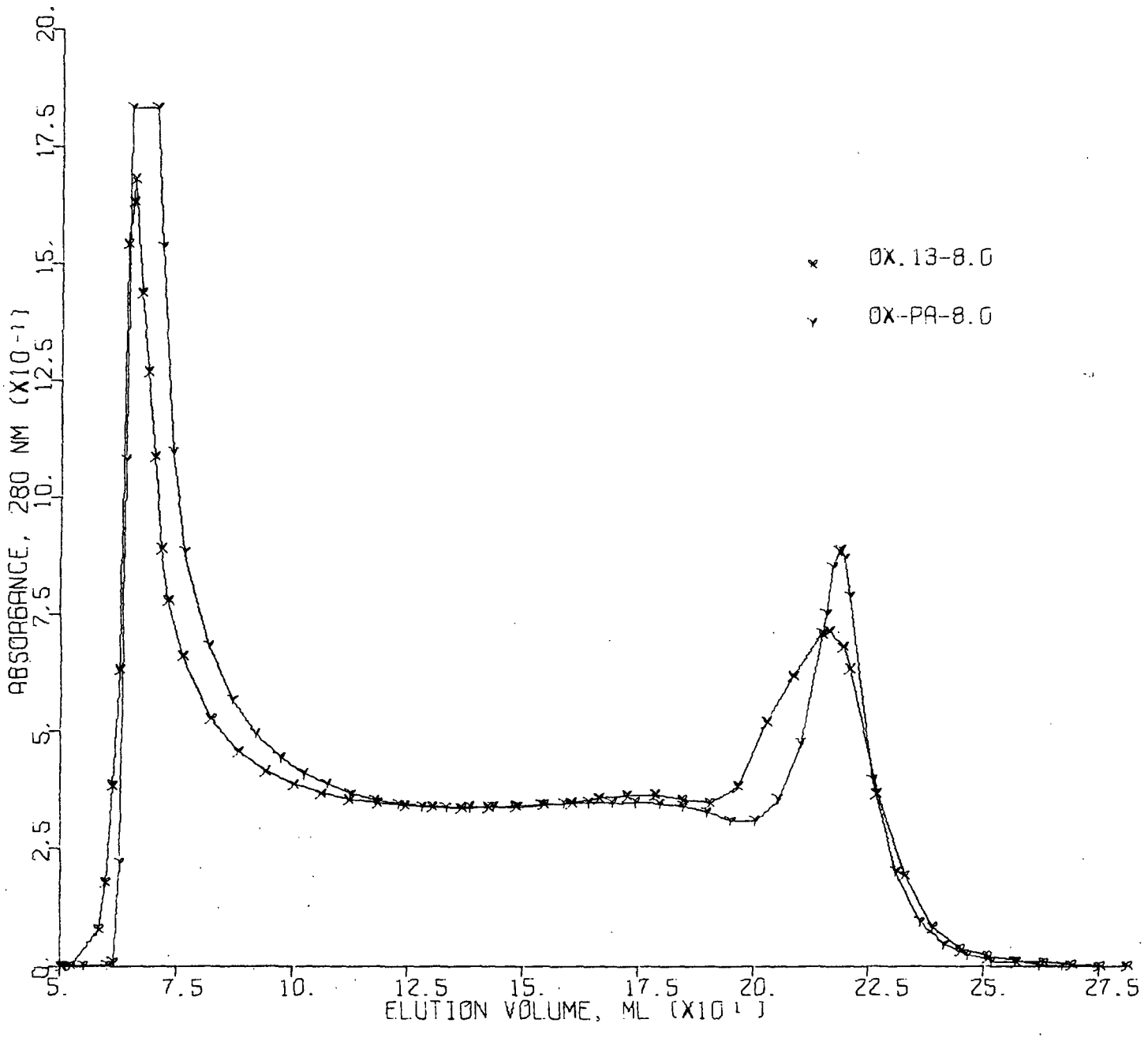


Figure 43. Comparison of Molecular Weight Distributions of OX.13-8.0 and OX-PA-8.0 Samples Determined by Chromatography on a Column of Sephadex G-75, with Formamide as Solvent

Oxidation of the PAA-modified lignin with oxygen in 0.13N Na_2CO_3 and comparison of chemical analyses with DL oxidized with oxygen in 0.13N Na_2CO_3 indicated only minor differences in the reactivity of the PAA-modified lignin. Interpretation of results was complicated by the fact that a portion of the initial alkali charge was effectively neutralized by carboxylic acids formed in the PAA pretreatment. Results may be summarized as follows:

1. Methoxyl contents decreased with increasing time of oxidation for both oxidations.
2. Direct UV spectra indicated that in both oxidations, there was an increase in absorptivity at 260 and 300 nm, with the absorptivity at 280 nm remaining essentially constant. In view of the lack of a decrease in absorptivity at 280 nm as would have been expected due to loss of methoxyl and phenolic hydroxyl, it was proposed that the changes in the UV spectra supported the formation of biphenyl linkages in the lignin.
3. Infrared spectra indicated that a major reaction in both O/A oxidations was an increase in carboxylic acids. Determination of carboxylic acid groups by potentiometric titration indicated a rapid initial increase in carboxyl content. The PAA-modified lignin contained a higher carboxyl content in the SM sample, but the carboxyl content of the unmodified lignin approached that of the modified lignin after 8.0 hours of oxidation.
4. In both oxidation series phenolic hydroxyl was rapidly consumed in the initial stages of reaction.
5. In both oxidation systems carbonyl was rapidly consumed in the initial stages of reaction.

6. Molecular weight distributions in both oxidations showed similar trends. There was rapid formation of a high molecular weight fraction early in the oxidation, followed by degradation of the high molecular weight portion to low molecular weight products with time of oxidation. Comparison of the molecular weight distributions of the two oxidation series after 8.0 hours of oxidation indicated that the high molecular weight portion of the unmodified lignin was much smaller than that of the corresponding PAA-modified lignin sample. This result was thought to be due to more extensive cross-linking of the PAA-modified lignin on charging the reactor with oxygen and alkali. The greater cross-linking was attributed to greater mobility of the lignin which arose as a consequence of lignin fragmentation on oxidation of α-carbonyls with PAA.

EXPERIMENTAL

PREPARATION OF MATERIALS

WATER

Water used in the lignin isolation and oxidation procedures was triply distilled, the second distillation from alkaline permanganate. Deionized, distilled water was obtained from a commercial Pyrex still. The second distillation was made from a solution of 0.02% $KMnO_4$ and 0.05% KOH to remove organic impurities. The final distillation was additive-free. Specific conductance of the water was 1.5×10^{-6} $ohm^{-1}cm^{-1}$, determined by Steve Tse with a calibrated conductivity cell.

1,4-DIOXANE

1,4-Dioxane used in the isolation of the lignin was purified by the method of Fieser (101) in order to eliminate peroxides. A mixture of 1.5 liters of 1,4-dioxane (Baker Analyzed reagent grade), 27 ml of concentrated HCl, and 200 ml of triply distilled water was combined in a 3-neck, 2-liter round-bottom flask and refluxed for 12 hours under nitrogen. Following cooling, the mixture was treated with KOH pellets and the aqueous layer separated. The dioxane was then poured into a 2-liter round-bottom flask and refluxed with sodium metal for 12 hours. Finally, the 1,4-dioxane was distilled fractionally from the sodium metal under a nitrogen atmosphere.

PEROXYACETIC ACID

Peroxyacetic acid (PAA) was prepared by the hydrogen peroxide (50%, obtained from the Combined Locks, WI mill of Appleton Papers) oxidation of acetic acid with sulfuric acid as catalyst, using the procedure of the FMC Corporation (102) as described by Farrand (98). PAA and hydrogen peroxide

were analyzed as described by Sully and Williams (99). The peroxyacetic acid was obtained at a concentration of 36.10% PAA with 0.78% H_2O_2 (w/w).

LOBLOLLY PINE DIOXANE LIGNIN

Two plantation-grown bolts of loblolly pine were obtained from Dr. William Gladstone, Weyerhaeuser Company, Hot Springs, Arkansas. The bolts were approximately 6-7 inches in diameter by 4-ft long and counted 17 growth rings. One bolt was stored. The second bolt was debarked with a scraper and cut on a round saw into 1-inch thick disks. Twenty-eight knot-free disks were hand-cut into chips using a guillotine blade, with a chip size of about 1/8-inch by 1 inch. The center two inches of each disk was discarded. A total of 31 pounds of chips was collected and air dried. The air-dried chips were ground on a Wiley mill to pass a Number 5 screen (20 mesh). Fifteen pounds of air-dried wood meal was collected and stored in a refrigerator at 40°F for further processing.

The wood meal was extracted in a large Soxhlet extractor placed on a 12-liter flask containing 7 liters of acetone. Acetone was used rather than the more commonly used alcohol-benzene solution to avoid the possibility of alcohol reaction with the lignin. A batch of about 900 g of wood meal was extracted for 12 hours. The spent acetone was replaced after 12 hours, again after a second 12 hours, and a third time after an additional 24 hours. A 100-ml aliquot of acetone from each extraction period was removed, evaporated, dried under vacuum over Drierite, weighed, and used to calculate total extractives. Results are summarized in Table XXVIII. Based on these results, it was concluded that acetone extraction for periods longer than 48 hours would not significantly increase extractives removal. Thus, extractions of succeeding batches of wood meal were carried out for a total period of 48 hours with replacement of acetone after the first 12 hours.

TABLE XXVIII

EXTRACTIVES REMOVAL AS A FUNCTION OF TIME

Time of Extraction, hr	Total Weight of Extractives Removed, g	% of Total Extractives Removed During Period
12	20.8	92.4
24	1.26	5.6
48	0.40	1.7

The average percent extractives removed, based on o.d. wood meal, was 3.3%. This extractive level was somewhat higher than values reported previously by Max (103) for alcohol-benzene (2.76%) and for ether (1.83%) and by Rydholm (104) for ether (2.5%) extraction of loblolly pine. In order to verify that ether extractable material was not left in the wood meal, a sample of the extracted meal was further extracted with ether for 6 hours. Upon evaporation and drying, less than 0.1% additional material was found to have been extracted. Following acetone extraction, the wood meal was air dried overnight and stored at 40°F.

The dioxane lignin (DL) was prepared according to the procedure of Pepper, et al. (35-37) with slight modifications. Aliquots of approximately 100 g of extracted wood meal (calculated on an o.d. basis) were ground on a micro-Wiley mill to pass a 60-mesh screen and placed in a 3-neck, 2-liter round-bottom flask equipped with magnetic stirrer, reflux condenser, dropping funnel, and nitrogen inlet-vacuum outlet. The flask was evacuated and vacuum maintained for 15 minutes. An oxygen-free solution of 1000 ml of 0.2N HCl in freshly prepared dioxane/water (9/1) was introduced through the dropping funnel into the flask, maintaining vacuum. After about one-half of the solution had been added, nitrogen gas was introduced, vacuum shut off, and the remainder of the solution added. The mixture was then stirred

and heated slowly (time to temperature, 1/2 hr) to 90°C. The mixture was refluxed (88 ± 2°C) for 1 hour, then allowed to cool. After cooling the suspension was filtered in a glove bag under nitrogen atmosphere. The wood meal was washed with 250 ml of neutral dioxane/water (9/1). The filtrate was next neutralized with sodium bicarbonate and concentrated on a rotary evaporator to an oil. The concentrate was added through a funnel under vacuum to an oxygen-free, 1% sodium sulfate solution (1250 ml) to yield a precipitate. The precipitate was isolated by centrifugation, then washed 3 times with triply distilled water and centrifuged. The isolated precipitate was shell frozen, freeze-dried, and stored under vacuum over P_2O_5 in the dark at 40°F.

The procedure was modified from the original method of Pepper, et al. (35-37) by use of 60-mesh wood meal rather than 20-mesh and by increasing the amount of wood meal and dioxane/water/HCl solution processed in each run by fourfold.

Total yield of dried lignin was 136.1 g (20.6% of the Klason lignin). Initial attempts to homogenize the lignin by reprecipitation of the combined lignin from freshly prepared dioxane/water (9/1) gave an inhomogeneous precipitate, based on elemental analyses. Therefore, lignin homogenization was achieved by combining all of the dried lignin in a mortar and grinding together to a fine powder with a pestle. The homogenized lignin was then partitioned into polyethylene bottles and stored in a vacuum desiccator over P_2O_5 in the dark at 40°F until needed for further analysis.

PEROXYACETIC ACID-MODIFIED LIGNIN

Ten grams of DL was dissolved in 100 ml of acetic acid/water (9/1) to which was added sufficient PAA solution to give a molar ratio of 0.50 PAA/

lignin (lignin formula weight = 188.9). The total volume was adjusted to 200 ml with acetic acid/water (9/1) and the solution reacted at 25°C until PAA could no longer be detected by the procedure of Sully and Williams (99). PAA was completely consumed after reacting for 52 hours. The reacted lignin was freeze-dried and stored under vacuum over P_2O_5.

Preliminary experiments on PAA-modified lignins were run as above, except that 1.0 g of DL dissolved in 10 ml of acetic acid/water (9/1) was reacted with appropriate amounts of PAA. Total volume of these solutions was adjusted to 20 ml with acetic acid/water (9/1).

LIGNIN OXIDATION WITH OYXGEN/ALKALI

Lignin oxidations were carried out in a 150-ml Teflon-lined reactor fitted with an alkali injection device, an internal thermocouple, an oxygen charging line, and a gas sampling line. The reactor system is described in Appendix V.

Prior to addition of lignin and alkali, the reactor was preheated in a water bath at 80°C for 1.0 hour. The lignin sample (1.0 g) and triply distilled water (50 ml) were placed in the bottom of the reactor. A weighed amount of alkali (0.6841 g or 2.6498 g Na_2CO_3, corresponding to 0.13N or 0.50N, respectively) was placed in the Teflon injector tube which was attached to the lid of the reactor on the oxygen charging line. The reactor was then flushed with nitrogen, sealed, and brought to temperature (120°C) in the oil bath (described in Appendix V). Time to temperature was 75 minutes. The lignin slurry was magnetically stirred during this time to minimize adhesion of the lignin to the surface of the Teflon liner. Once at temperature, oxygen was introduced to the reactor for 1 minute at a pressure of 121 psig (0.618 MPa, 89.7 psia, @ 120°C, Appendix VI). The internal temperature of the reactor

increased by about 0.5°C upon charging, then returned to 120°C after about 15 minutes. The plug in the bottom of the injector tube was blown out by the oxygen and introduced the alkali to the reactor with the oxygen.

Preparation of the SM sample and 0.0-hour sample was described previously under Nomenclature and General Procedures.

Reaction was terminated after the desired period of time by reducing the oxygen pressure to atmospheric and immersing the reactor in cold water. The reactor was opened and the liquor and undissolved lignin (if any) removed by pipeting into a beaker.

The final reaction pH was measured and unreacted base estimated by titration with 0.1N HCl to pH 7.0. The sample pH was next adjusted to 3.0 by addition of 1.0N HCl and the solution bubbled with nitrogen and stirred for 3.0 hours to remove CO_2. Finally, the pH was adjusted to pH 9.0 by addition of 1.0N NaOH and the sample freeze-dried.

ANALYTICAL PROCEDURES

KLASON LIGNIN

Klason lignin was determined by TAPPI Standard Method T 13, corrected for ash in the wood. The method as used in this study was modified to the extent that no extraction was made other than the original acetone extraction.

ASH

Ash content of the DL was determined as follows. An accurately weighed sample of the lignin (0.2 g) (of known moisture content) was placed into a tared porcelain crucible. The sample was heated slowly with access of air. Finally, the sample was ignited at 550°C for 2 hours. The sample was then placed in a desiccator under vacuum over P_2O_5 to cool, then weighed.

Sulfated ash was determined on oxidized lignin samples in order to calculate sodium content. To the ashed sample prepared as above was added 1.0 ml of concentrated sulfuric acid. Excess sulfuric acid was driven off by heating the crucible overnight on a hot plate. Once the sample was dry, it was placed in a muffle furnace, heated gradually, and finally ignited at 750°C for 2 hours. The ignited sample was placed in a vacuum desiccator over P_2O_5 to cool, then weighed.

METALS

Metal contents reported in Table XXX of Appendix II were determined by the Analytical Group of The Institute of Paper Chemistry (IPC) with a 1.5 m Bausch & Lomb Emission Spectrograph. Metal contents in Tables XXXI and XXXII of Appendix II were determined by the Analytical Group of IPC with a Perkin-Elmer Model 305 Atomic Absorption Spectrophotometer.

CARBOHYDRATE

Carbohydrate content was determined by the Analytical Group of IPC by the method of Borchart and Piper (48).

ELEMENTAL ANALYSIS

Analyses for carbon and hydrogen were performed by Chemalytics, Inc. of Tempe, Arizona. Oxygen content was computed by difference.

METHOXYL

Methoxyl content was determined by Institute Method 18, which is based on the conversion of methyl from the methoxyl groups to methyl iodide by the action of hydriodic acid. The original method specified the addition of 3 g of phenol to each sample to promote complete conversion of methoxyl to methyl-

iodide. However, the phenol apparently inhibited stoichiometric conversion of the methoxyl, as analyses of model compounds gave low values. A search of the literature revealed that Samsel and McHard (105) had likewise observed that phenol had a tendency to depress alkoxy values in their analyses of vanillin and ethyl cellulose. The TAPPI Standard Method T 209 for determination of methoxyl in pulps specified addition of propionic anhydride in order to promote complete reaction of the methoxyl groups. Analyses of model compounds indicated that propionic anhydride solutions gave results in agreement with theoretical values. Therefore, analyses of lignin samples were run with addition of 3 ml of propionic anhydride.

ULTRAVIOLET SPECTROSCOPY

Samples for UV analysis were prepared in a manner similar to the procedure of Arseneau and Pepper (37). Ultraviolet spectra were determined with a Cary Model 15 Recording Spectrophotometer, using 1 cm silica cells. Scan speed was 30 nm/minute.

Samples for direct UV spectra were prepared by dissolving 35 mg of lignin in 5 ml of freshly prepared 1,4-dioxane. The solution was then brought to 100 ml in a volumetric flask with methanol/water (4/1). Five milliliters of this stock solution was pipeted into a 50-ml volumetric flask and brought to volume with methanol/water (4/1).

Samples for UV difference spectra were prepared by pipeting 10 ml of the stock solution described above into a 50-ml volumetric flask containing 5 ml of 1N NaOH or 1N HCl and bringing to volume with methanol/water (4/1). Spectra were run by placing the basic solution in the sample beam and the acidic solution in the reference beam.

INFRARED SPECTROSCOPY

Infrared spectra were determined with a Perkin-Elmer Model 700 Spectrophotometer, using sodium chloride optics. Samples were prepared by grinding 1 mg of lignin with 100 mg of KCl and pressing into a pellet. Pellets were evacuated in the press for 5 minutes, then pressed at 20,000 psi for 5 minutes.

A borohydride-reduced sample of DL was prepared according to the method of Sarkanen, et al. (64). A sample of DL (25 mg) was dissolved in a mixture of 2 ml of 95% ethanol and 1 ml of 0.1N NaOH under nitrogen. Sodium borohydride (20 mg) and water (2 ml) were added and the solution was allowed to react for 2 days at room temperature under nitrogen. After 2 days the solution was acidified with 0.1N HCl to pH 4 and isolated by centrifugation. After washing twice with distilled water and isolation by centrifugation, the lignin was freeze-dried.

CARBON-13 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

The ^{13}C NMR spectra were taken on solutions of two of the lignin preparations (DL and OX-PA-SM) in deuteroacetone/deuterium oxide (9/1) with a Jeol Model FX100 FT NMR Spectrometer, operating at 25.05 MHz by the Fourier-Transform technique. Concentration of the samples was 20%. Spectra were run using 10-mm NMR tubes containing solution volumes of 2.5 ml. Three hundred blocks of 1000 transients were accumulated in the frequency domain accumulation mode. The spectrophotometer was locked to the $-CD_3$ groups of the acetone and the central signal of the acetone multiplet at 29.8 ppm was used as internal reference. The carbonyl and methyl groups of the acetone were allowed to overflow. Shift values quoted are given in the TMS scale relative to the acetone standard (29.8 ppm).

PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

Samples for PMR spectroscopy were acetylated by placing 25 mg of lignin in 1 ml of water-free pyridine. The DL, SM, and 0.0 samples were readily soluble, but the oxidized samples remained virtually insoluble. One-half milliliter of dimethylformamide was added to the pyridine in order to aid solubilization of the oxidized samples (82), followed by 1 ml of acetic anhydride. The samples were covered and allowed to stand at room temperature for 24 hours. Samples were precipitated by addition of the reaction solution to 25 ml of ice water. The acetylated lignin was taken up in 15 ml of chloroform and the aqueous layer washed twice with 5-ml aliquots of chloroform. The chloroform wash was combined with the initial 15 ml of chloroform. The chloroform was next washed 3 times with 10 ml of 1.0N HCl and once with 15 ml of distilled water. The chloroform solution was dried overnight over anhydrous sodium sulfate, then concentrated to a sirup under reduced pressure on a rotary evaporator, and finally placed under vacuum for 24 hours at room temperature. The sirup was taken up in 100% deuterated chloroform (Stohler Isotope Chem., Rutherford, NJ) containing a trace of TMS and transferred to a 5-mm NMR tube.

PMR spectra of the acetylated lignins were taken on the chloroform solutions with a Jeol Model FX100 FT NMR Spectrometer equipped with a dual carbon-hydrogen probe. The spectrometer was locked to the -CD- signal of the chloroform and TMS was used as internal reference. Shift values quoted are given in the TMS scale.

The DL, SM, and 0.0 samples gave very good spectra with 200 pulses. However, the oxidized lignin samples required 1000 pulses to give comparable signal to noise ratios, indicating that there was less acetylated product in the oxidized samples.

PHENOLIC HYDROXYL BY UV DIFFERENCE SPECTROSCOPY

Phenolic hydroxyl content of the lignin was determined from the UV difference spectrum by the method of Wexler (83), as modified by Arseneau and Pepper (37). The procedure depends upon measurement of the peak height at 250 nm, which has been found to be directly related to the concentration of ionized phenolic hydroxyl groups. The base line was set by drawing a line from the absorbance minimum at 229 nm to the minimum at 280 nm (Fig. 44). Phenolic hydroxyl content was then calculated by multiplying the absorptivity at 250 nm by an empirical factor of 0.192 determined by Wexler (83), based on the study of a number of softwood lignin model compounds.

PHENOLIC HYDROXYL BY PMR SPECTROSCOPY

Phenolic hydroxyl content was determined from the integral of the PMR spectra of the acetylated lignins by the procedure of Morohoshi and Sakakibara (79). Phenolic hydroxyl was computed from the integral of the phenyl acetoxy protons (2.40-2.19 ppm) by equating the total integral to the hydrogen content determined from the elemental analysis.

CARBONYL

Carbonyl content was determined according to the hydroxylamine hydrochloride method of Gierer and Soderberg (86). Approximately 100 mg of lignin was weighed exactly and dissolved in 20 ml of 95% ethanol. The solution pH was adjusted to 4.00 with a Corning Model 12 Research pH Meter. The pH of a solution of 40 ml of hydroxylamine hydrochloride (3.450 g/liter) in 95% ethanol was likewise adjusted to 4.00. The two solutions were combined at time zero in a 100-ml beaker covered with parafilm. The solution was stirred magnetically at 35°C under a nitrogen atmosphere. The solution level was maintained constant by periodic addition of absolute ethanol to replace that

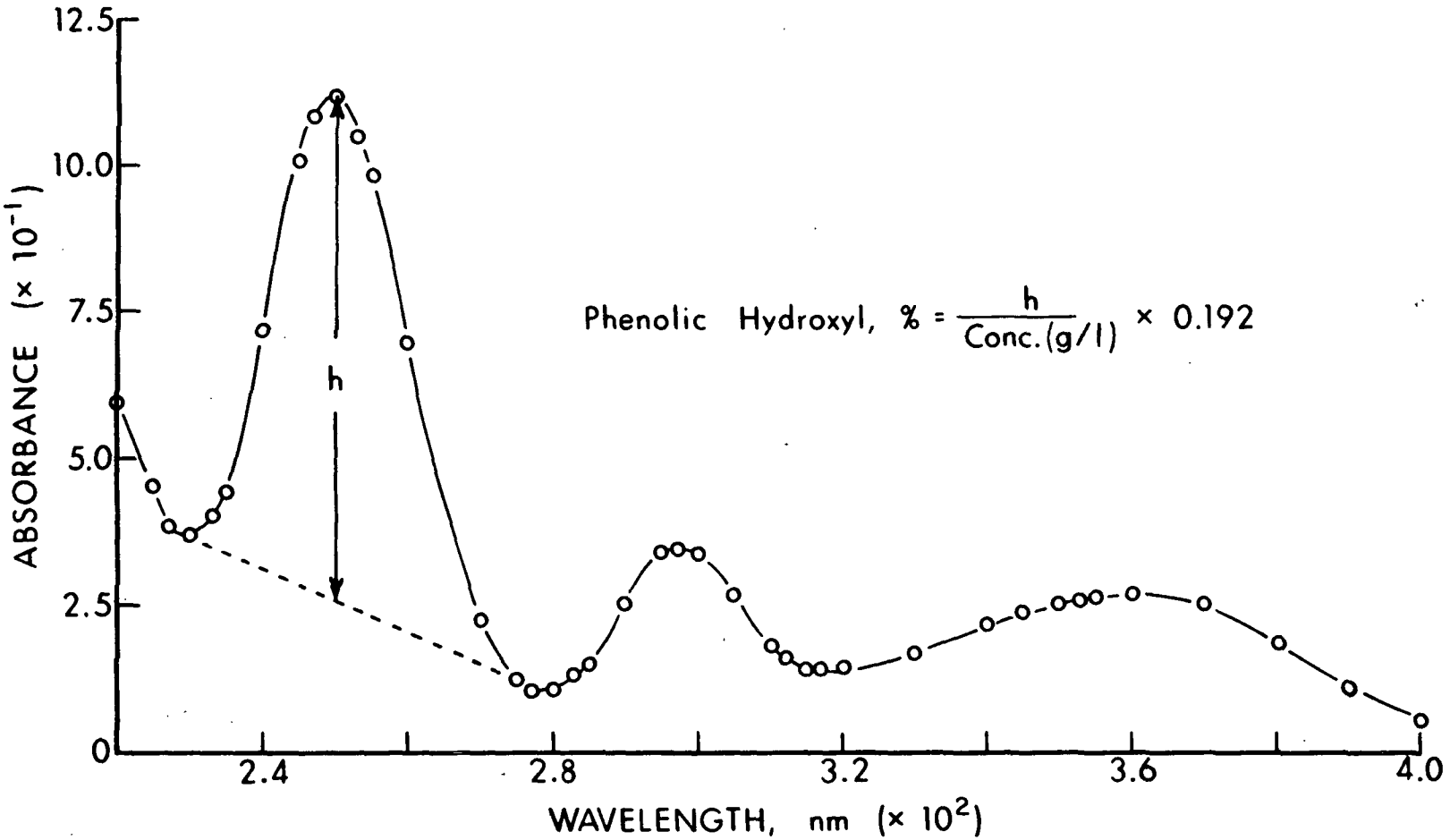


Figure 44. UV Difference Spectrum of Loblolly Pine Dioxane Lignin Illustrating the Method for Calculation of Phenolic Hydroxyl

lost by evaporation. At varying periods of time the solution pH was adjusted to 4.00 with 0.0100N NaOH and the milliequivalents of base required to titrate plotted as a function of time of reaction. The carbonyl content was computed by extrapolation of the pH time plot back to time zero (Fig. 45). The positive slope of the plot at long reaction times was due to the spontaneous degradation of the reagent (86).

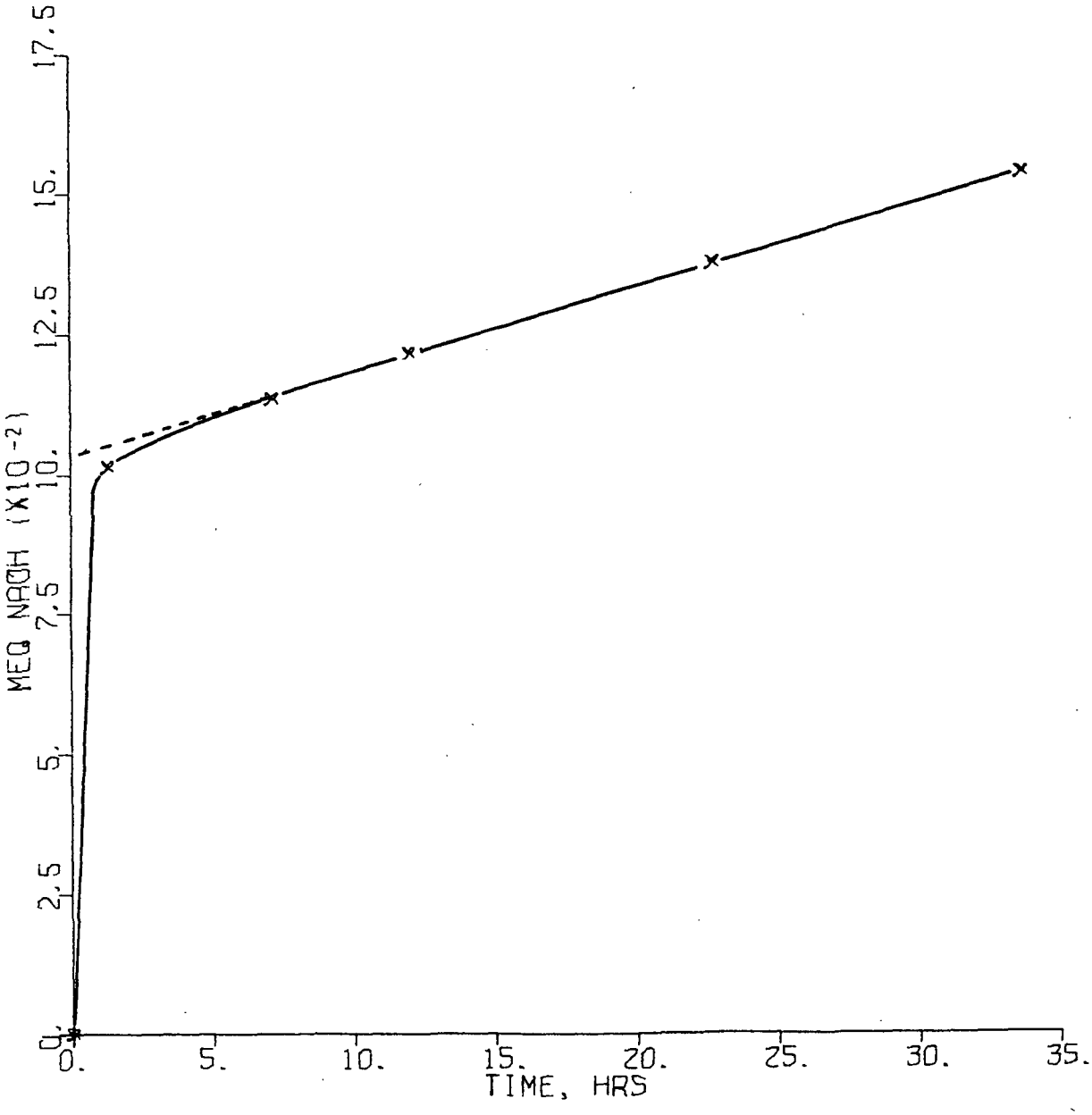


Figure 45. Method for Calculation of Carbonyl Content

CARBOXYL

Carboxyl content was measured by potentiometric titration using a Corning Model 12 Research pH Meter. A 0.1-g sample of lignin was accurately weighed into a 250-ml beaker to which was added 100 ml of triply distilled water and sufficient sulfuric acid (7×10^{-2}N) to bring the pH below 3.0. The solution was then titrated with 0.0100N NaOH, allowing 5 minutes between incremental additions for the solution to equilibrate. The milliequivalents of carboxyl was calculated as the milliequivalents of base required to titrate from pH 3.80 to the inflection point at pH 7.40, corrected for the milliequivalents of base required to titrate a blank consisting of an equivalent amount of sulfuric acid (7×10^{-2}N) over the same pH range (Fig. 46).

MOLECULAR WEIGHT DISTRIBUTION

Sephadex G-50, Superfine (15 g) was swollen for 24 hours in reagent grade formamide (Fisher Scientific) (500 ml). The resulting slurry was added to a borosilicate glass column (2.5 x 85 cm) fitted at the base with a sintered glass disk to give a resin bed 75 cm in length. The top of the column was protected with a circular fiberglass filter disk. Each sample, consisting of 50 mg of lignin dissolved in 3 ml of formamide, was washed onto the column with several 3-ml aliquots of formamide. The column was eluted with formamide at a rate of 0.24 ml/min.

Columns (2.5 x 50 cm) were also prepared with Sephadex G-75, Superfine and Sephadex G-100, Superfine, as above. Total bed length of both columns was 45 cm. The Sephadex G-75 and G-100 columns were eluted with formamide at rates of 0.11 and 0.05 ml/min, respectively.

Absorbance of the eluate was monitored on a continuous basis at 280 nm using the Cary Model 15 Recording Spectrophotometer in the synchronous mode

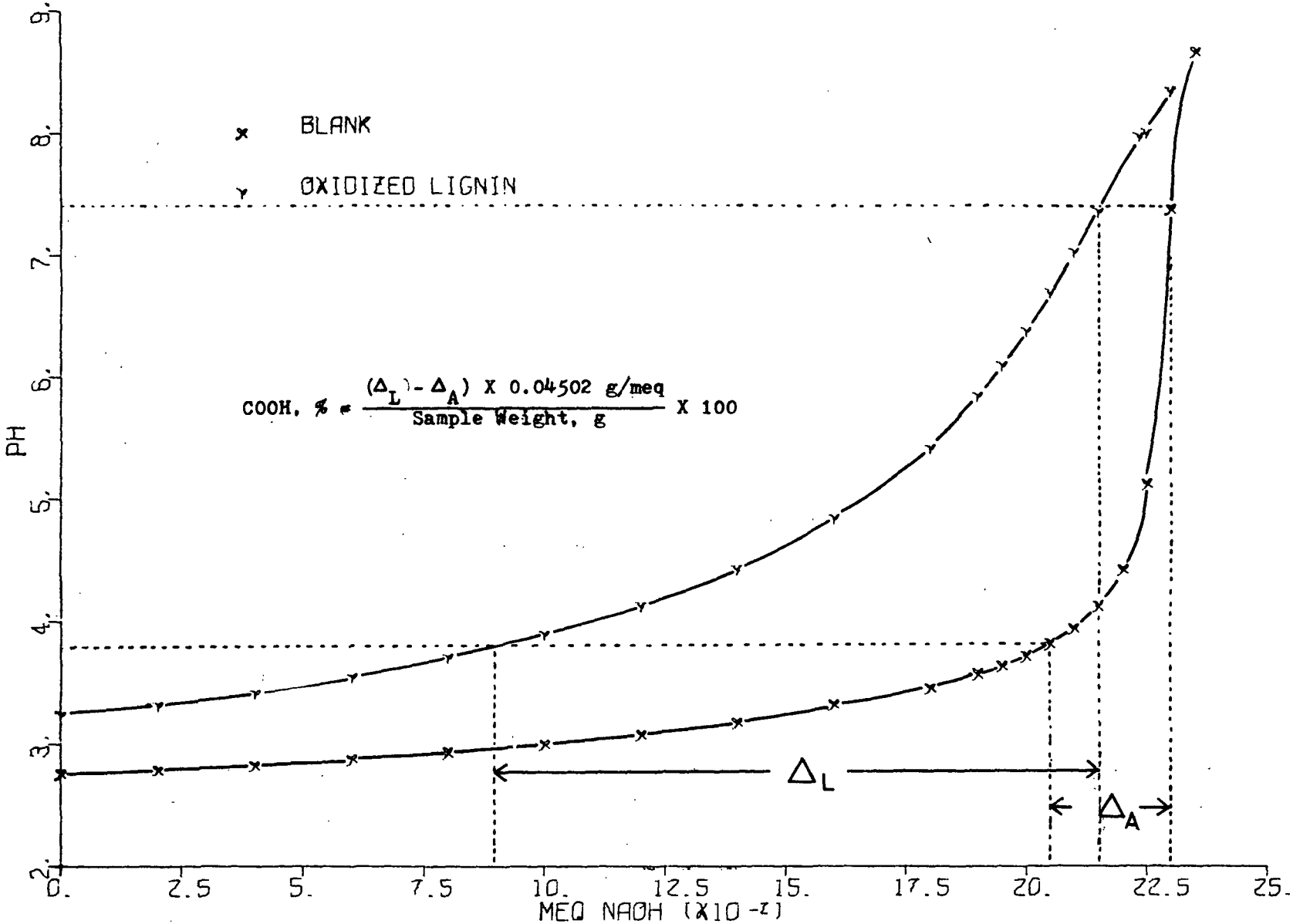


Figure 46. Method for Calculation of Carboxyl Content

with the scan speed shift lever set at X1. Spectra were obtained using matched silica flow-through cells with a path length of 0.1 cm.

WEIGHT AVERAGE MOLECULAR WEIGHT

The weight average molecular weight was determined by the sedimentation equilibrium technique with a Beckman Model E Analytical Ultracentrifuge. Determinations were made in reagent grade formamide (Fisher Scientific). Molecular weight and density calculations are discussed in Appendix IV.

SUGGESTIONS FOR FURTHER RESEARCH

This investigation has examined changes in the chemical properties of an isolated softwood lignin upon oxidation with molecular oxygen in sodium carbonate solutions. Results indicated that the lignin underwent extensive cross-linking upon charging the reactor with oxygen/alkali to give a high molecular weight lignin which was subsequently degraded to low molecular weight products. Based on model compound studies by other workers, functional groups which are important in the initiating reactions of oxygen/alkali degradation are phenolic hydroxyls and α-carbonyls. Levels of both of these functionalities were greatly reduced during the first hour of oxidation with oxygen/alkali, suggesting that secondary reactions predominate at longer reaction times.

Peroxyacetic acid has been found to increase the reactivity of the hardwood lignin in fiberized red maple toward degradation with oxygen in sodium carbonate solutions (8). However, treatment of a softwood with peroxyacetic acid by these workers failed to increase the lignin reactivity.

Similarly, modification of the isolated softwood lignin used in this investigation with peroxyacetic acid failed to increase the reactivity of the lignin toward degradation with oxygen in sodium carbonate solutions. It would be of interest to perform a study to parallel the present investigation using an isolated hardwood lignin to attempt to identify the reasons for the different reactivity of hardwood and softwood lignins.

Karna (47) studied molecular weight distributions of isolated softwood lignins at pH 10 and 14 and observed that, while oxidation with molecular oxygen at pH 10 led to formation of a high molecular weight lignin fraction, oxidation at pH 14 produced no high molecular weight fraction. He therefore

postulated that the mechanism of lignin degradation at pH 10 was different than the mechanism for degradation at pH 14. A study such as the present one, carried out at pH 14, would be expected to help elucidate the apparent differences in mechanism.

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ABBREVIATIONS

C_9 unit = guaiacylpropane

CEL = cellulolytic enzyme lignin

DL = dioxane lignin

DMF = dimethylformamide

GPC = gel permeation chromatography

$\bar{M}_w$ = weight average molecular weight

MWL = milled wood lignin

N = normal

OX.13 = dioxane lignin oxidized with oxygen in 0.13N Na_2CO_3

OX.50 = dioxane lignin oxidized with oxygen in 0.50N Na_2CO_3

OX-PA = peroxyacetic acid-modified dioxane lignin oxidized with oxygen in 0.13N Na_2CO_3

PAA = peroxyacetic acid

TMS = tetramethylsilane

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APPENDIX I

MOISTURE CONTENTS BASED ON OVEN-DRY WEIGHT

This appendix contains moisture contents for the DL and oxidized lignins calculated from the formula:

$$\text{Moisture content, \%} = \frac{(P_2O_5/\text{vacuum dry wt.}) - (\text{o.d. wt.})}{(\text{o.d. wt.})} \times 100,$$

where o.d. weight was determined following heating at 110°C for 24 hours.

TABLE XXIX

MOISTURE CONTENTS BASED ON OVEN-DRY WEIGHT

Sample Number	Moisture Content, %	
DL-1	5.73	Average = 5.64%
-2	6.09	
-3	5.73	
-4	5.28	
-5	5.05	
-6	5.97	
OX.13-SM	1.43	
-0.0	1.01	
-0.5	4.19	
-1.0	2.67	
-2.0	9.87	
-4.0	7.67	
-8.0	5.57	
OX.50-SM	3.47	
-0.5	2.18	
-1.0	2.28	
-2.0	4.75	
-4.0	7.20	
-8.0	3.20	
OX-PA-SM	5.55	
-0.0	8.95	
-0.5	6.76	
-1.0	12.10	
-2.0	4.89	
-8.0	16.60	

APPENDIX II

METAL CONTENTS

TABLE XXX

METAL CONTENT OF DIOXANE LIGNIN DETERMINED BY EMISSION SPECTROSCOPY

Metal	Analysis[a,b], % Sample 1[c]	Sample 2[c]
Aluminum	0.0044	0.0044
Barium	0.00058	0.00054
Boron	0.00073	0.00090
Calcium	0.015	0.021
Copper	0.0066	0.0069
Iron	0.017	0.026
Lead	0.00090	0.0012
Magnesium	0.0042	0.0052
Manganese	0.014	0.014
Silicon	0.011	0.012
Sodium	1.0	1.0
Total	1.07	1.10

[a] Based on o.d. weight.

[b] Average of duplicate determinations.

[c] Sample numbers refer to separate aliquots of DL.

TABLE XXXI

METAL CONTENT OF DIOXANE LIGNIN DETERMINED BY ATOMIC ABSORPTION SPECTROSCOPY

Metal	Analysis[a,b], %
Cobalt	0.0013
Copper	0.0087
Iron	0.015
Magnesium	0.0034
Manganese	0.0130
Nickel	<0.0082

[a] Based on o.d. weight.

[b] Based on a single determination using perchloric acid digestion.

Metal contents presented in Table XXXII were based on percent weight in the o.d. sample. Since ash contents of the OX.13 and OX-PA samples were about 50% while the ash content of the OX.50 samples was about 75%, the percent metal content of the OX.50 samples was effectively diluted by the higher ash, relative to the OX.13 and OX-PA samples. Thus, on a lignin basis the magnesium content of the OX.13-0.0 sample was (0.0029/0.5314) 0.0055%, while the magnesium content of the OX.50-0.0 sample was (0.0012/0.2249) 0.0053%.

TABLE XXXII

METAL CONTENTS OF OXIDIZED LIGNINS DETERMINED BY ATOMIC ABSORPTION SPECTROSCOPY

	Analysis[a,b], %					
Metal/Sample	OX.13-0.0	OX.13-2.0	OX.50-0.0	OX.50-4.0	OX-PA-0.5	OX-PA-8.0
Cobalt	0.0081	0.0040	0.0027	0.0032	0.0023	0.0051
Copper	0.0062	0.0058	0.0032	0.0120	0.0150	0.0049
Iron	<0.0061	0.0062	<0.0032	<0.0038	0.0041	<0.0042
Magnesium	0.0029	0.0022	0.0012	0.0017	0.0022	0.0019
Manganese	0.0076	0.0064	0.0028	0.0027	0.0060	0.0045
Nickel	<0.0270	<0.0180	<0.0140	<0.0170	<0.0120	<0.0190

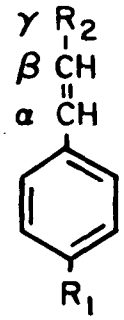
[a]Based on o.d. weight.

[b]Based on a single determination using perchloric acid digestion.

APPENDIX III

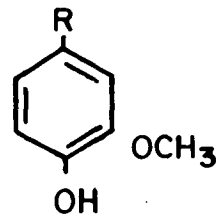
MODEL COMPOUNDS USED FOR ASSIGNMENT OF PEAKS IN THE ^{13}C NMR SPECTRUM OF LIGNIN (73)

The ^{13}C NMR spectra of model compounds listed in this appendix were studied by Ludemann and Nimz (72,73) and used to assign peaks in the ^{13}C NMR spectrum of spruce lignin. The compounds are listed here to facilitate discussion of ^{13}C spectra of the DL and PAA modified lignins used in this investigation. Compound numbers correspond to numbers in parentheses in Table VI.



1

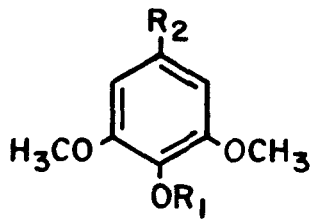
	R_1	R_2
1a	H	CHO
1b	OH	COOH



2

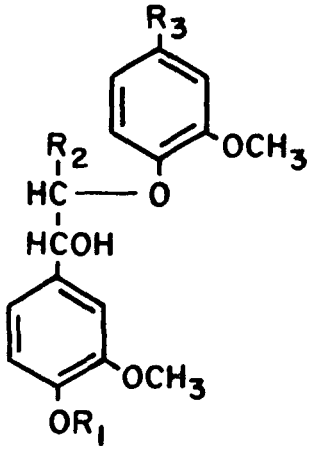
2a: R=H
b: R=CH_3
c: R=CHO
d: R=CH=CH-CH_3
e: R=CH=CH-COOH

Appendix III (Continued)



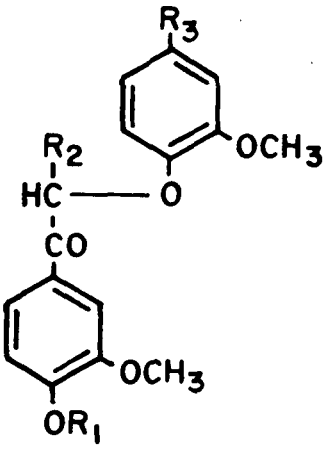
3

	R_1	R_2
3a	H	CH_2OH
b	H	COOH
c	H	CHO
d	CH_3	CHO
e	$COCH_3$	CHO
f	$CH_2C_6H_5$	CHO
g	CH_3	CH=CH-COOH



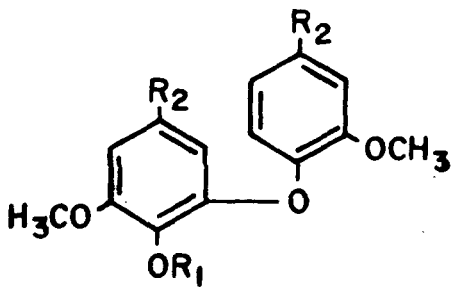
4

	R_1	R_2	R_3
4a	H	H	CH_3
b	H	CH_3	CH_3
c	$CH_2C_6H_5$	H	CH_2OH
d	H	CH_2OH	$(CH_2)_3OH$



5

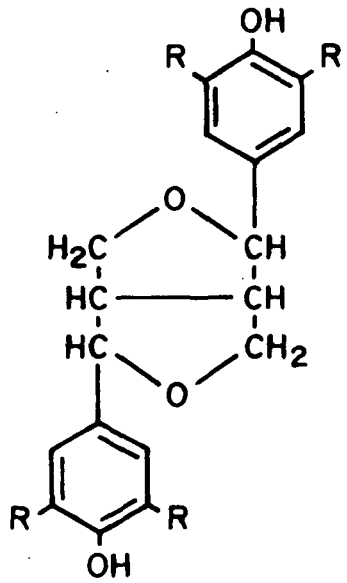
	R_1	R_2	R_3
5a	H	CH_3	CH_3
b	$CH_2C_6H_5$	CH_3	CHO
c	$CH_2C_6H_5$	CH_2OH	CHO



6

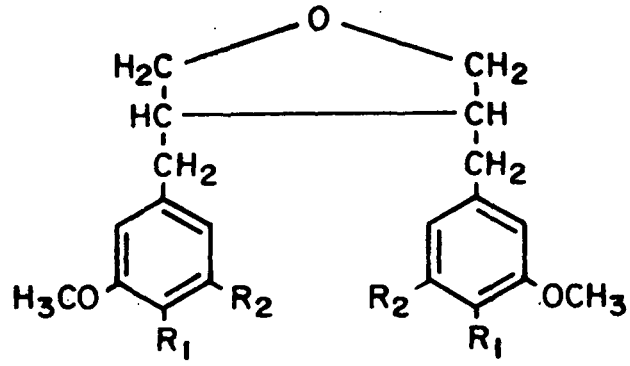
	R_1	R_2
6a	H	COOH
b	CH_3	CHO

Appendix III (Continued)



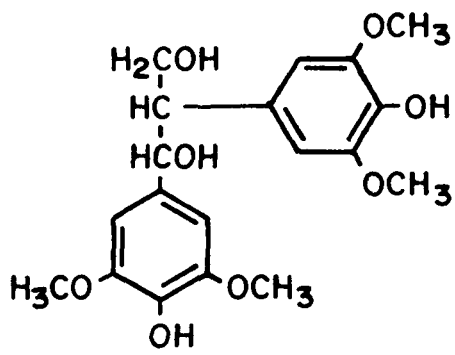
7

7a: R = H
b: R = OCH_3

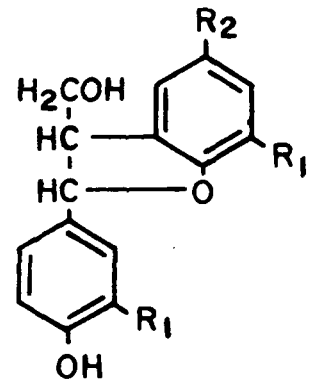


8

	R_1	R_2
8a	OH	H
b	$OCOCH_3$	OCH_3



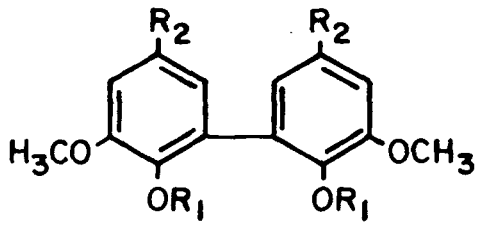
9



10

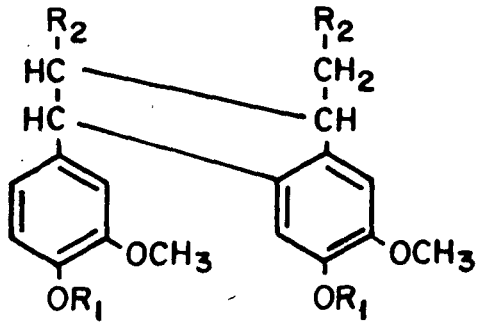
	R_1	R_2
10a	H	$CH=CH-CH_2OH$
b	OCH_3	$CH_2-CH_2-CH_2OH$
c	OCH_3	$CH=CH-CH_2OH$

Appendix III (Continued)



11

	R_1	R_2
11a	H	COOH
b	H	$CH_2-CH_2-CH_3$
c	H	CH=CH-COOH
d	$COCH_3$	$CH_2-CH_2-COOCH_2C_2H_5$



12

	R_1	R_2
12a	H	CH_3
b	H	$COOC_2H_5$
c	$COCH_3$	$COOC_2H_5$
d	CH_3	COOH

APPENDIX IV

DETERMINATION OF MOLECULAR WEIGHT BY SEDIMENTATION EQUILIBRIUM ANALYSIS

Determination of weight average molecular weights of lignin samples was very difficult due to the polydispersity of the material. Therefore, several variations of the sedimentation equilibrium method were tried in an attempt to obtain meaningful values. Analyses were run by John Carlson of the Institute staff. The sedimentation equilibrium technique depends on the measurement of the solute concentration distribution in a centrifugal field under conditions of thermodynamic equilibrium. The three variations of sedimentation equilibrium applied to the two lignin samples studied are known as the conventional, short column, and meniscus depletion methods. Each method will be described briefly. A more extensive review of ultracentrifuge techniques has been written by Chervenka (106).

All sedimentation runs were made with reagent grade formamide (Fisher Scientific Co.) at 25°C. The density of the formamide at 25°C was determined with a pycnometer to be 1.12537 by the method of Bauer (107). The density of a solution of DL in formamide at a concentration of 0.2416% (w/w) was 1.12588. The partial specific volume, $\bar{v}$, was 0.701, calculated by the relationship:

$$\bar{v} = \frac{1}{d_o} - (1/c)\ (d-d_o)/d_o$$

where d_o = density of formamide at 25°C

d = density of solution at 25°C

c = solution concentration

In the conventional sedimentation equilibrium method the measurement is made on a fluid column length of 3 to 5 mm in the centrifuge cell, with rotor

speeds sufficiently low that the solute concentration at the meniscus is not zero, and with the entire contents of the cell at sedimentation equilibrium. In order to compute the molecular weight, the concentration of solute throughout the cell must be determined at sedimentation equilibrium. In addition, the measurement temperature, rotor speed, solvent density, and partial specific volume of the sample must be accurately known. The concentration distribution at sedimentation equilibrium was determined by schlieren optics. The weight average molecular weights of the DL and the high molecular weight fraction of the OX.50-2.0 sample (Fig. 31) were estimated by the conventional sedimentation equilibrium method. The partial specific volume of the DL (0.701) was assumed to be valid for the OX.50-2.0 sample, as the concentration of solute in this sample was too low to permit determination of the partial specific volume.

Weight average molecular weights were calculated by the conventional sedimentation equilibrium method according to the equation:

$$\bar{M}_w = \frac{2RT}{(1 - \bar{v}\rho)\omega^2(b^2 - a^2)} \times \frac{\Delta c}{c_o}$$

where R = gas constant

T = temperature in °K

$\bar{v}$ = partial specific volume

ρ = solvent density

ω = rotor speed

a,b = cell constants

Δc = concentration distribution

c_o = solution concentration

Results are summarized in Table XXXIII for the DL sample and in Table XXXIV for the OX.50-2.0 sample.

TABLE XXXIII

WEIGHT AVERAGE MOLECULAR WEIGHT OF DIOXANE LIGNIN DETERMINED BY CONVENTIONAL SEDIMENTATION EQUILIBRIUM

c_o, %	ω, rpm	$\bar{M}_w$	
0.05	36000	10720	determined with a 30-mm cell
0.05	30000	11710	
0.05	24000	15130	
0.05	52000	11720	determined with a 12-mm cell
0.05	48000	12700	
0.05	44000	13470	
0.05	40000	14410	
0.05	36000	15460	
0.05	32000	17800	
0.05	28000	17540	
0.05	24000	18470	

The dependence of $\bar{M}_w$ on rotor speed is evident. At high rotor speeds the molecular weight of the DL appeared to approach 11,000 in both cells.

TABLE XXXIV

WEIGHT AVERAGE MOLECULAR WEIGHT OF OX.50-2.0 HIGH MW FRACTION DETERMINED BY CONVENTIONAL SEDIMENTATION EQUILIBRIUM

c_o, %	ω, rpm	$\bar{M}_w$
0.065	20000	95820
0.065	18000	109520
0.065	14000	112670
0.065	12000	135490
0.065	8000	152110

The dependence of $\bar{M}_w$ on rotor speed was even more pronounced for the higher molecular weight sample. It was felt that the preferred method for running the higher molecular weight sample was by meniscus depletion.

The meniscus depletion method is essentially the same as the conventional method discussed above, except that the centrifuge is operated at a speed sufficiently high that all solute is sedimented out of the region of the cell near the meniscus. An added advantage of the method is that number average, as well as weight average molecular weight, may be calculated. Determination of the molecular weight of the OX.50-2.0 sample by the meniscus depletion method indicated a weight average molecular weight of 78,000 and a number average molecular weight of 64,200 (c_o = 0.065%; $\bar{v}$ = 0.701; ω = 30,000 rpm).

The short column sedimentation equilibrium technique is a modification of the conventional method which enables running samples in 3 hours, rather than the 24 hours normally required to reach equilibrium in the conventional method. The short column method employs a fluid column length of 1 mm or less, compared to the 3 to 5 mm required in the conventional method. The short column method is considered to be less accurate, but may be useful for survey purposes. Molecular weight is calculated from the relationship,

$$\bar{M}_w = \frac{RT}{(1 - \bar{v}\rho)\omega^2} \times \frac{1}{R_{mid}\, c_o} (dc/dr)_{mid}$$

where R = gas constant

T = temperature in degrees Kelvin

$\bar{v}$ = partial specific volume

ρ = solvent density

R_{mid} = radial midpoint of the image of the sample fluid column

c_o = solution concentration

$(dc/dr)_{mid}$ = concentration distribution at the radial midpoint

Results from the short column equilibrium sedimentation analysis of the DL are summarized in Table XXXV.

TABLE XXXV

WEIGHT AVERAGE MOLECULAR WEIGHT OF DIOXANE LIGNIN DETERMINED BY SHORT COLUMN SEDIMENTATION EQUILIBRIUM

c_o, %	ω, rpm	$\bar{M}_w$
0.255	32000	9850
0.255	40000	6766
0.10	32000	9952
0.10	40000	6580

APPENDIX V

THE REACTOR SYSTEM

REACTION BOMB AND COVER

The basic reactor design was originally conceived by McCloskey (108) and modified by Millard (109). The reactor design used in the investigation was essentially the same as one used by Millard (109), differing only in dimensions. The internal chamber of the reactor was completely Teflon lined in order to prevent contamination of the reaction liquor with metal. Details of reactor construction are illustrated in Fig. 47.

The reactor pot was constructed of brass, fitted internally with a machined Teflon insert. The reactor cover was likewise made of brass and fitted with a Teflon shield (1/32 inch thickness; Eagle Supply and Plastics, Inc., Appleton, WI). The cover was bolted to the flange on the reactor pot to provide a gas-tight seal.

The reactor was equipped with one sampling and one pressurizing line, constructed of Teflon tubing (0.031 inch ID x 0.061 inch OD; Laboratory Data Control, Inc., Riviera Beach, Fl). Each of the two lines was attached to an on-off valve (Cheminert On-Off Valve, Chromatronix, Laboratory Data Control, Inc.). A pressure-tight seal was obtained on the inside of the reactor by drawing the Teflon tubing through a Kel-F plug (Eagle Supply and Plastics, Inc., Appleton, WI) drilled such that the diameter was slightly undersized. The Kel-F plug was screwed into the underside of the reactor cover against the Teflon shield to obtain a pressure-tight seal. For a detailed diagram of the fittings, refer to Millard (109). The tubing outside of the reactor was supported by passing it through a 1-ft length of 1/8 inch OD stainless

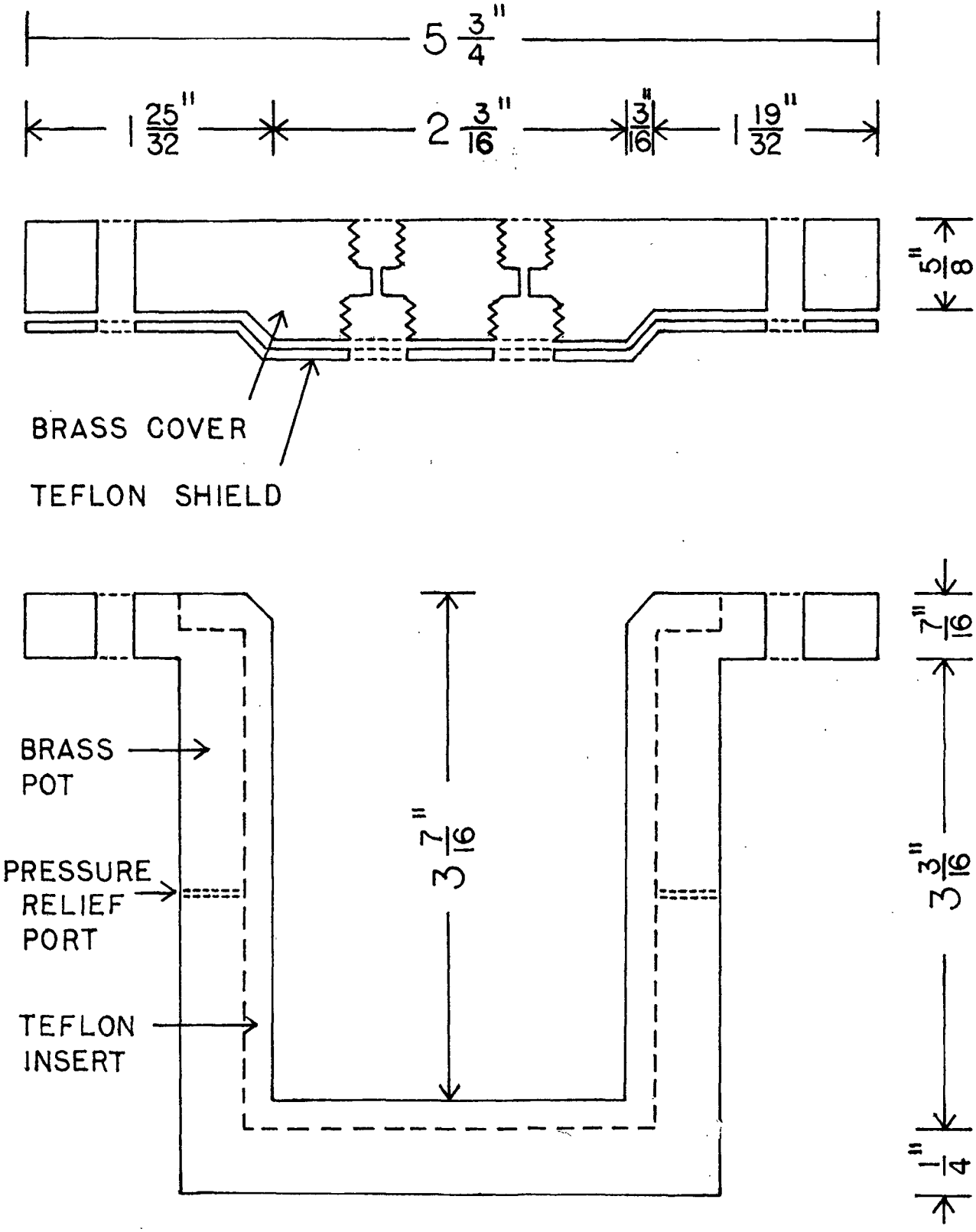


Figure 47. Details of Reactor Construction

steel tubing attached to the reactor cover with Swagelock tube fittings (Badger Valve & Tube Fitting Corp., Wauwatosa, WI).

The reactor was also equipped with an inconel sheathed, grounded, copper-constantan quick disconnect thermocouple (Omega Engineering, Inc., Stamford, CT) to monitor the temperature inside the reactor. The thermocouple was encased in a sealed 1/8 inch thick Kel-F sleeve inside the reactor to prevent contact of the oxidation liquor with the bare metal. The Kel-F sheath was attached to a Kel-F plug which was screwed into the underside of the reactor cover against the Teflon shield to obtain a pressure-tight seal. On the top of the reactor cover the thermocouple was held in place with a Swagelock fitting.

Alkali was introduced to the reactor at the time of oxygen charging from an injection tube (Fig. 48). The injection tube was originally intended to be used for introduction of the powdered lignin to the alkali solution, as had been done by Renard, et al. (110). However, in the course of bringing the reactor to temperature, the glass transition temperature of the lignin was passed and the lignin tended to adhere to the wall of the injector tube (Appendix VII).

OIL BATH AND TEMPERATURE CONTROL

The oil bath was modelled after one described by Brandon (111). The shell of the bath was twin-walled stainless steel with calcium silicate insulation between the walls. The reactor was bolted to a hand crank which was used to raise and lower the reactor into the bath by releasing a locking gear. Bath temperature was controlled with a Polyscience Model 73 Immersion Circulator (0-1000 w; Polyscience Corp., Niles, IL) fitted with a thermostat preset to 120°C (J. L. Stortz Div., PSG Industries, Inc., Perkaskie, PA). The power supply cord to the immersion circulator ran to an Over-Temp Guard set at 130°C (Instruments for Research and Industry, Cheltenham, PA).

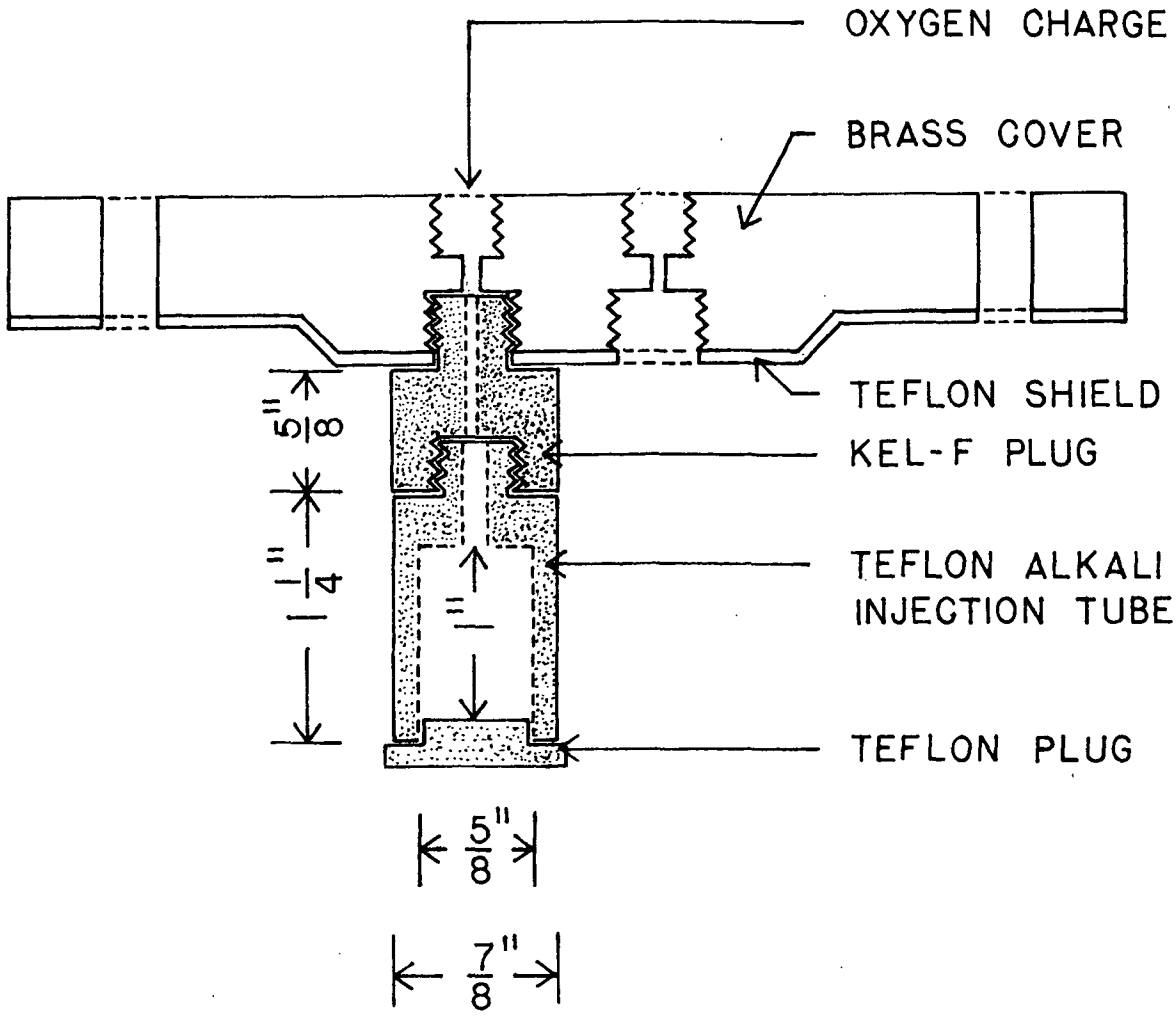


Figure 48. Details of Alkali Injection Device

The temperature inside the reactor was monitored with an inconel-sheathed, grounded, copper-constantan quick disconnect thermocouple (Omega Engineering, Inc., Stamford, CT) referenced to a copper-constantan thermocouple reference junction (T, Charles T. Gamble Ind., Riverside, NJ). The reference junction was plugged into a flatbed recorder (Leeds & Northrup Speedomax XL Recorder, Model 628) which permitted continuous monitoring of the reactor temperature.

Magnetic stirring of the reactor was provided by an air driven "Mag-Jet" (Matheson Scientific, Inc.). The stirrer was clamped directly to the bottom of the reactor prior to lowering the reactor into the oil bath.

APPENDIX VI

CALCULATION OF INITIAL OXYGEN PRESSURE

This appendix contains the detailed calculation of the actual oxygen pressure in the reactor at time zero.

Preheat reactor to 80°C and flush with N_2, assume gas temperature to be 60°C --- 14.7 psi N_2 (@ 60°C)

Bring reactor to 120°C --- 14.7 psi N_2 x 393°K/333°K = 17.3 psi N_2

Vapor pressure of water @ 120°C = 28.795 psia

Correct for vapor pressure lowering by 0.6841 g Na_2CO_3

From CRC, 48th edition, p. E1 ---

0.5 g mole Na_2CO_3 = 14.3 mm lowering

$$\frac{0.6841 \text{ g } Na_2CO_3}{106 \text{ g } Na_2CO_3/\text{mole}} \times \frac{1000 \text{ ml/liter}}{50 \text{ ml}} = 0.1291 \text{ mole } Na_2CO_3/\text{liter}$$

Assume lowering is linear -

$$\frac{0.1291 \text{ mole } Na_2CO_3}{0.5 \text{ mole } Na_2CO_3} \times 14.3 \text{ mm} = 3.692 \text{ mm (@ 100°C)}$$

At 100°C vapor pressure of water = 14.696 - (14.696 x 3.692/760)

= 14.625 psia

Corrected vapor pressure @ 120°C = 28.795/14.696 x 14.625 psi

= 28.656 psi

At 120°C introduce O_2 @ 121 psig

Actual pressure = 121 + 14.7 = 135.7 psia

Initial oxygen pressure @ 120°C = 135.7 - 28.7 - 17.3 = 89.7 psia

It was next of interest to estimate the number of moles of oxygen available per equivalent weight of lignin. The volume of the reactor was estimated to be 147 ml by measurement of the volume of water contained with the cover on.

Since there was 50 ml of alkali solution in each run, the gas volume was 97 ml. Thus, moles of oxygen were given by:

$$n_i = \frac{89.7 \text{ psi}/14.7 \text{ psi/atm} \times 0.097 \text{ liter}}{0.08205 \text{ liter-atm/deg.-mole} \times 393°K} = 0.0184 \text{ mole } O_2$$

The ratio of moles of O_2 to equivalent weight of lignin was:

$$\frac{\text{moles } O_2}{\text{equivalent wt. lignin}} = \frac{0.0184/1.0 \text{ g}}{188.2 \text{ g/equiv.}} = 3.46$$

APPENDIX VII

GLASS TRANSITION TEMPERATURE

Renard, et al. (110), in their study of the rate of lignin degradation with oxygen in alkaline medium, used a stainless steel injection tube to introduce the lignin to the oxidation liquor with the oxygen at temperature. The obvious advantage of this design was that there was no opportunity for alkaline degradation of the lignin prior to introduction of the oxygen. A similar device, constructed of Teflon, was used in the present investigation (Fig. 48) with the intent of introducing the lignin and the oxygen together to the alkaline liquor once the reactor was at temperature (120°C).

However, instead of entering the liquor as a finely divided powder upon charging with oxygen, the lignin went in as a tacky ball. The problem was attributed to the fact that the glass transition temperature, T_g, of the lignin was lower than 120°C. In the softened state the lignin was very tacky and tended to adhere to any surface it contacted. The problem was circumvented by placing the lignin and 50 ml of triply distilled water in the bottom of the reactor and a weighed amount of alkali in the injection tube. It was observed under these conditions that the lignin would remain finely divided if the solution was rapidly stirred while the reactor was brought to temperature.

Literature on glass transition phenomena associated with lignin has been reviewed by Goring (112). Goring (93) determined a softening temperature, T_s, for a number of isolated lignins (Table XXXVI) by a thermal softening technique. The T_s was felt to be representative of the T_g for these samples. Two properties of the lignin which affected the T_s were identified: first, the higher the molecular weight of the lignin, the higher the T_s; and second, for a given lignin, the higher the moisture content, the lower the T_s.

TABLE XXXVI

THERMAL SOFTENING TEMPERATURES FOR SPRUCE DIOXANE LIGNINS (93)

Lignin Sample	$\bar{M}_w$	Dry T_s, °C	Moisture Uptake, %	Moist T_s, °C
Spruce DL 1	4300	127	7.7	72
Spruce DL 2	7400	136		
Spruce DL 3	33000	146	7.8	92
Spruce DL 4	85000	172		

In a subsequent study by Ramiah and Goring (113) the T_g of a spruce DL ($\bar{M}_w$ = 15,000) was estimated to be 80-85°C by thermal expansion using dilatometry.

The T_g of a 6-mg sample of DL from this investigation was estimated to be 64°C (moisture content = 5.6%) with a Perkin-Elmer Model DSC-1B Differential Scanning Calorimeter. Scanning rate was 10°C/minute at 5 mv and a range setting of 4.

Operation of the differential scanning calorimeter is based on temperature control of two similar sample holders in the sample holder assembly. A reference sample is heated at a preset rate of temperature increase. The unknown sample is heated at the same rate by applying additional heat to the sample as required. The differential voltage requirement between the reference and unknown samples is sensed by a thermocouple and recorded. The glass transition for the lignin was an endothermic process and thus required additional heat input to maintain the reference temperature. The T_g was estimated by drawing tangents through the base line and the rise in heat capacity (114) as illustrated in Fig. 49.

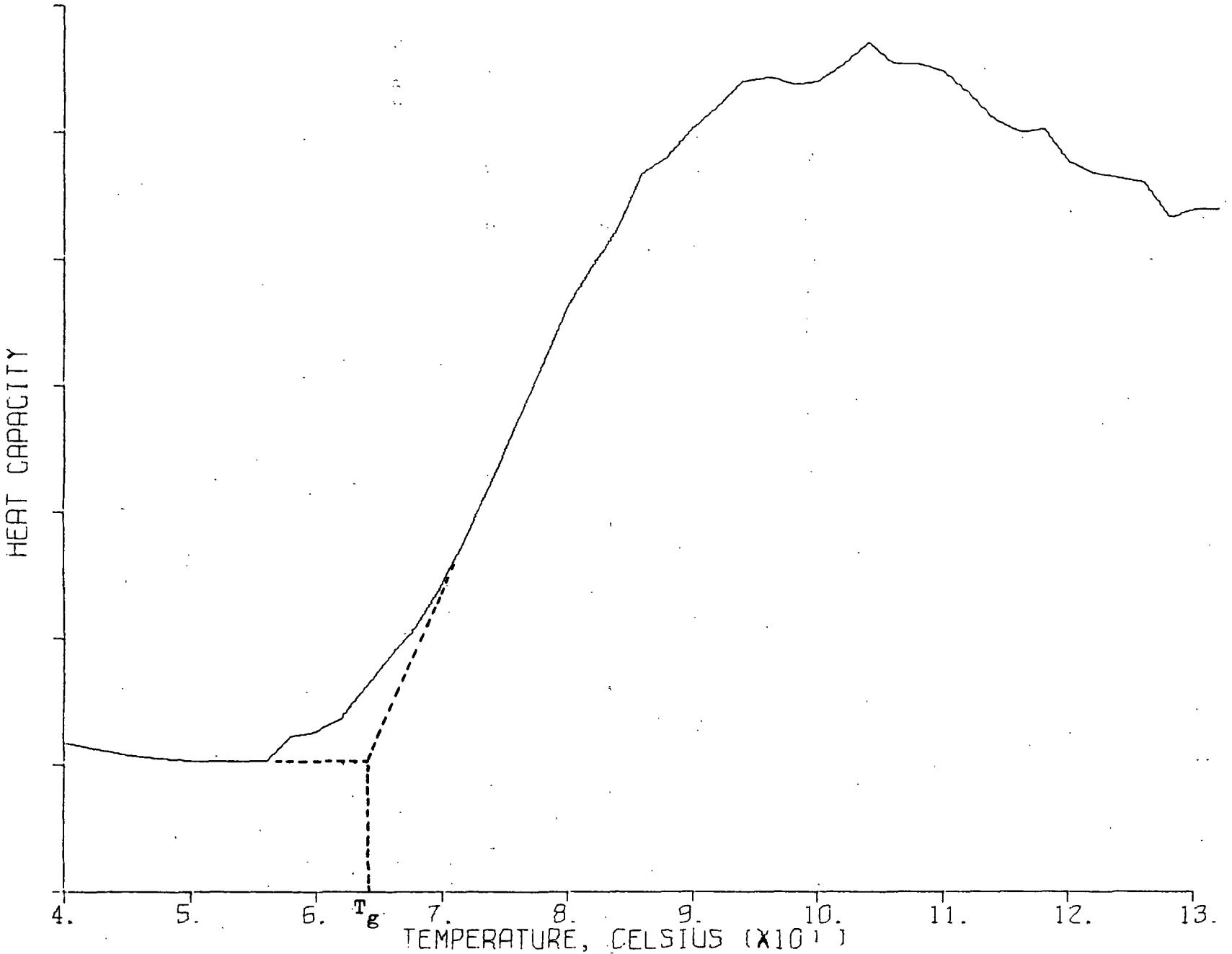


Figure 49. Method for Determination of Glass Transition Temperature (T_g) by Differential Scanning Calorimetry

APPENDIX VIII

RATE OF OXYGEN SOLUBILITY IN SODIUM CARBONATE SOLUTIONS

In order to be able to have an idea of the time necessary for the alkali solution to become saturated with oxygen following oxygen charging of the reactor, a series of determinations of dissolved oxygen in sodium carbonate solutions were made using the pressurized system described by Green, et al. (115). Dissolved oxygen was determined by use of a modified Winkler procedure. Results are summarized in Table XXXVII.

TABLE XXXVII

CONCENTRATION OF DISSOLVED OXYGEN IN SODIUM CARBONATE SOLUTIONS

Time, min	Na_2CO_3, M/l	O_2 Conc., ppm
9	0.129	77.3
15	0.129	68.2
67	0.129	77.1
589	0.129	67.7
5	0.500	44.8
14	0.500	60.6
75	0.500	62.5

Due to the estimated 10% error associated with the method and the presence of nitrogen in the reactor, oxygen solubilities determined represent at best an estimation of the absolute concentration of oxygen in solution. However, based on these measurements, it appeared that the solution was saturated within 10 minutes of introduction of oxygen, a short time relative to the length of time of reaction.

No values for the solubility of oxygen in sodium carbonate solutions have been reported in the literature. However, concentrations determined

here are in the same range as values determined by Hearne (average O_2 concentrate = 80 ppm) (116) in 1.25N NaOH solutions at an O_2 pressure of 131 psig and 120°C.

APPENDIX IX

CARBON DIOXIDE ANALYSES FOLLOWING LIGNIN OXIDATION

Following oxidation of the lignin in the OX.13 series, two gas samples were collected from the reactor in 1.0-ml pushbutton valve syringes (Series A-2; Supelco, Inc., Bellefonte, PA). Gas samples (0.4 ml) were analyzed using a 6 ft x 1/8 inch stainless steel column packed with Carbosieve. The column was mounted for on-column injection in an Aerograph Model 200 Gas Chromatograph with a thermal conductivity detector. Helium was used as carrier gas at a flow rate of 20 ml/minute. Detector output was recorded with a Sargent Model SR Recorder at a chart speed of 4 inches/minute. After the oxygen peak came off the column at 45°C, a 30°C/minute column oven program was initiated to raise the temperature from 45 to 175°C, at which point the CO_2 peak came off. Detector and injector temperatures were both 190°C.

Peak areas were integrated on a Technicon Integrator/Calculator Model AAG. There was a significant base-line drift between the beginning and end of a peak. A correction was made for drift by drawing a line from the point of initial peak formation to a point 1 inch after the peak tail had leveled and using this line as the base line for integration. Peak area responses corresponding to an attenuation setting of 128×10^{-11} were calculated.

A calibration gas mixture of 10% CO_2 and 90% O_2 (Alltech Assoc., Arlington Heights, IL) was used for calculation of response factors and relative percentages of CO_2 and O_2 by the following relationships:

$$\text{Response factor (R.F.)} = \frac{\text{Area } CO_2 \text{ (std.)}}{\text{Area } O_2 \text{ (std.)}} \times \frac{90\%\ O_2}{10\%\ CO_2}$$

$$\%\ CO_2 = \frac{\%\ CO_2/\%\ O_2 \times 100}{\%\ CO_2/\%\ O_2 + \text{R.F.}} \qquad \%\ O_2 = \frac{100 \times \text{R.F.}}{\%\ CO_2/\%\ O_2 + \text{R.F.}}$$

Results from analyses of gas samples collected from the OX.13 oxidation series are presented in Table XXXVIII. The sample labelled syringe 1 was the first sample collected from the reactor in each case.

TABLE XXXVIII

CARBON DIOXIDE ANALYSES FOR OXIDATION SERIES OX.13

Syringe Number	Sample Number	O_2 Integral[a]	CO_2 Integral[a]	CO_2/O_2	Response Factor	O_2, %	CO_2, %
1	OX.13-0.5	165.5	6.75	0.0408		98.02	1.97
1	standard	75.2	17.75	0.2359	2.123		
2	OX.13-0.5	158	4.94	0.0312		98.49	1.51
1	OX.13-1.0	168.5	5.25	0.0312		98.49	1.51
1	standard	30.8	8.58	0.2786	2.507		
2	OX.13-1.0	170.2	11.42	0.0671		96.80	3.20
1	OX.13-2.0	146.2	24.38	0.1667		92.41	7.59
1	standard	142.8	34.88	0.2443	2.199		
2	OX.13-2.0	138.5	Off scale	--		--	--
1	OX.13-8.0	165	10.25	0.0621		97.03	2.97
1	standard	135	32.81	0.2431	2.187		
2	OX.13-8.0	101.5	Off scale	--		--	--

[a]Integrals were adjusted to an attenuation of 128×10^{-11}.

No carbon monoxide was observed in any of the gas samples. There appeared to be a tendency for the second gas sample removed from the reactor to have a higher CO_2 content than the first sample; in the case of the 2.0 hour and 8.0 hour samples, significantly so. The source of the problem was

not known. There was a substantial drop in pressure during withdrawal of the two gas samples. A possible explanation for the higher CO_2 content in the second sample was that the liquor was saturated with CO_2 and once the pressure was reduced above the liquor, the solution rapidly released CO_2, thus increasing the CO_2 concentration in the gas phase. Because of this problem, carbon dioxide was not analyzed in subsequent oxidation series.

APPENDIX X

EVALUATION OF ASH CONTENT

TABLE XXXIX

EVALUATION OF ASH CONTENT FOR OX.13 SAMPLES[a]

Sample Number	SM	0.0	0.5	1.0	2.0	4.0	8.0
A. Total organic added to reactor, g	0.9208	0.9190	0.9182	0.9163	0.9068	0.9177	0.9144
B. Total inorganic added to reactor, g	1.0292	0.8105	0.7570	0.8270	0.8232	0.8288	0.8473
C. Total weight added to reactor, g, A+B	1.9500	1.7295	1.6752	1.7433	1.7300	1.7465	1.7617
D. Calculated ash, % [B/(A+B)] x 100	52.78	46.86	45.19	47.44	47.58	47.45	48.10
E. Total freeze-dried weight recovered, g	1.8738	1.6552	1.6017	1.7419	1.6564	1.7118	1.6449
F. % of total weight recovered (E/C) x 100	96.09	95.70	95.61	99.91	91.02	98.01	93.37
G. Sodium added, g	0.3883	0.3157	0.3445	0.3652	0.3741	0.3677	0.4104
H. Sodium recovered,[b] g	lost	0.3024	0.2895	0.3579	0.3494	0.3745	0.3884
I. % of total sodium recovered (H/G) x 100		95.79	84.03	98.00	93.40	101.85	94.57

[a] All weights based on o.d. weight.

[b] Sodium content computed from sulfated ash.

TABLE XL

EVALUATION OF ASH CONTENT FOR OX.50 SAMPLES[a]

Sample Number	0.0	0.5	1.0	2.0	4.0	8.0
A. Total organic added to reactor, g	0.9110	0.9121	0.9110	0.9127	0.9076	0.9097
B. Total inorganic added to reactor, g	3.1399	3.0656	3.1422	3.0591	2.9997	2.8175
C. Total weight added to reactor, g (A+B)	4.0509	3.9777	4.0532	3.9718	3.9073	3.7272
D. Calculated ash, % [B/(A+B)] x 100	77.51	77.07	77.52	77.02	76.77	75.59
E. Total freeze-dried weight recovered, g	3.9782	3.9097	4.0043	3.9247	3.8610	3.7866
F. % of total weight recovered (E/C) x 100	97.86	98.29	98.79	98.81	98.81	101.59
G. Sodium added, g		1.2453	1.2877	1.3093	1.2905	1.2979
H. Sodium recovered,[b] g	N.D.	1.2045	1.2496	1.2638	1.2574	1.2540
I. % of total sodium recovered (H/G) x 100		96.72	97.04	96.52	97.43	96.62

[a]All weights based on o.d. weight.

[b]Sodium content computed from sulfated ash.

TABLE XLI

EVALUATION OF ASH CONTENT FOR OX-PA SAMPLES[a]

Sample Number	SM	0.0	0.5	1.0	2.0	8.0
A. Total organic added to reactor, g	0.9115	0.9099	0.9099	0.9090	0.9088	0.8972
B. Total inorganic added to reactor, g	0.8591	0.8841	0.8799	0.8323	0.8601	0.8810
C. Total weight added to reactor, g (A+B)	1.7706	1.7940	1.7898	1.7413	1.7689	1.7782
D. Calculated ash, % [B/(A+B)] x 100	48.52	49.28	49.16	47.80	48.62	49.54
E. Total freeze-dried weight recovered, g	1.6977	1.6398	1.7401	1.6124	1.7103	1.7247
F. % of total weight recovered (E/C) x 100	95.88	91.41	97.22	92.60	96.69	96.99
G. Sodium added, g	0.3623	0.3996	0.4038	0.3612	0.4230	0.4235
H. Sodium recovered[b], g	0.3450	0.3247	0.3845	0.3228	0.3913	0.4073
I. % of total sodium recovered (H/G) x 100	95.22	81.26	95.22	89.37	92.51	96.17

[a]All weights based on o.d. weight.

[b]Sodium content computed from sulfated ash.

Comparison of percent of total weight recovered with percent of total sodium recovered in Tables XXXIX, XL, and XLI gave a measure of the accuracy of the calculated ash. In general, agreement between the two values was within 1 to 2%.